PCT

(51) International Patent Classification 6 :		(11) International Publication Number: WO 95/02604
C07H 19/167, A61K 31/70	A1	(43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/US94/07835

(22) International Filing Date: 13 July 1994 (13.07.94)

(30) Priority Data:
091,109 13 July 1993 (13.07.93) US
163,324 6 December 1993 (06.12.93) US

(71) Applicant: THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICESNational Institutes of Health [US/US]; Office de Technology Transfer, Box OTT, Bethesda, MD 20892-9902 (US).

(72) Inventors: JACOBSON, Kenneth, A.; 116506 Fulham Street, Silver Spring, MD 20902 (US). GALLO-RODRIGUEZ, Carola; Avenue Santa Fé 2533 8°A, RA-1425 Buenos Aires (AR). VAN GALEN, Philip, J., M.; Titus Brandsmaplein 40, NL-5342 EP Oss (NL). VON LUBITZ, Dag, K., J., E.; 6329 Dorset Drive, Alexandria, VA 22310 (US). JEONG, Heaok, Kim; 5603 Alderbrook Court #104, Rockville, MD 20851 (US).

(74) Agents: KILYK, John, Jr. et al.; Leydig, Voit & Mayer, Ltd., Suite 4900, Two Prudential Plaza, Chicago, IL 60601-6780 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A3 ADENOSINE RECEPTOR AGONISTS

(57) Abstract

The present invention provides N^6-benzyladenosine-5'-N-uronamide and related substituted compounds, particularly those containing substituents on the benzyl and/or uronamide groups, and modified xanthine ribosides, as well as pharmaceutical compositions containing such compounds. The present invention also provides a method of selectively activating an A3 adenosine receptor in a mammal, which method comprises acutely or chronically administering to a mammal in need of selective activation of its A3 adenosine receptor a therapeutically effective amount of a compound which binds with the A3 receptor so as to stimulate and A3 receptor-dependent response.

1

A_3 ADENOSINE RECEPTOR AGONISTS

This application is a continuation-in-part of
copending U.S. patent application serial no. 08/163,324,
filed December 6, 1993, which, in turn, is a
continuation-in-part of copending U.S. patent application
serial no. 08/091,109, filed July 13, 1993 and now
abandoned.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to A_3 adenosine
receptor agonists and methods of selectively activating
an A_3 adenosine receptor in a mammal. The present
invention also relates to methods of treating various
medical disorders with A_3 adenosine receptor agonists.

BACKGROUND OF THE INVENTION

Adenosine receptors, belonging to the superfamily of
the G protein-coupled receptors, are generally divided
into two major subclasses, A_1 and A_2, on the basis of the
differential affinities of a number of adenosine receptor
agonists and antagonists for the receptors, their primary
structures, and the secondary messenger systems to which
they couple. Thus, A_2 receptors, which can be further
subdivided into A_{2a} and A_{2b}, stimulate adenylate cyclase,
whereas A_1 receptors may couple to a variety of secondary
messenger systems, including those involved in the
inhibition of adenylate cyclase, the inhibition or
stimulation of phosphoinositol turnover, the activation
of guanylate cyclase, the activation of potassium influx,
and the inhibition of calcium influx (van Galen et al.,
Med. Res. Rev., 12, 423-471 (1992), and Jacobson et al.,
J. Med. Chem., 35, 407-422 (1992)).

Recently, a novel adenosine receptor was identified
on the basis of its primary structure and cloned from rat
brain (Zhou et al., Proc. Natl. Acad. Sci. U.S.A., 89,
7432-7436 (1992)) and rat testis (Meyerhof et al., FEBS

2

<u>Lett.</u>, <u>284</u>, 155-160 (1991)). The putative transmembrane
domains of the novel adenosine receptor, which has been
designated the A_3 receptor, show 58% identity with the
canine A_1 receptor and 57% with the canine A_{2a} receptor.
5   Like the A_1 receptor, the A_3 receptor is negatively
coupled to adenylate cyclase (Zhou et al., <u>supra</u>).

The potential utility of A_1- and A_2-selective agents
in therapeutic applications has been limited by
accompanying side effects, given the ubiquitous nature of
10   the A_1 and A_2 receptors. The distribution of the A_3
receptor, by contrast, is fairly limited, being found
primarily in the central nervous system (CNS) (Zhou et
al., <u>supra</u>), brain, testes (Meyerhof et al., <u>supra</u>), and
immune system, where it appears to be involved in the
15   modulation of release from mast cells of mediators of the
immediate hypersensitivity reaction (Ramkumar et al., <u>J.</u>
<u>Biol. Chem.</u>, <u>268</u>, 16887-16890 (1993)). The limited
distribution of the A_3 receptor provides a basis for
predicting that A_3-selective compounds may be more useful
20   than A_1- and A_2-selective compounds as potential
therapeutic agents. It is believed that A_3-selective
compounds will have utility in the therapeutic and/or
prophylactic treatment of cardiac disease, infertility,
kidney disease, and CNS disorders.

25   Few ligands for this novel receptor have been
reported. Some non-selective N^6-substituted adenosine
derivatives have been described as agonists for the A_3
receptor, including APNEA (N^6-2-(4-aminophenyl)ethyl-
adenosine), which has been used successfully as a
30   radioligand in its iodinated form (Zhou et al., <u>supra</u>).
Typical xanthine and nonxanthine A_1 and A_2 receptor
antagonists, however, do not appear to bind to this
receptor (Zhou et al., <u>supra</u>).

Thus, there remains a need for A_3-selective
35   agonists. The present invention seeks to provide such
compounds, as well as methods of using these compounds to
selectively activate the A_3 receptor in mammals, and

3

pharmaceutical compositions comprising such compounds.
These and other objects and advantages of the present
invention, as well as additional inventive features, will
be apparent from the description of the invention
5   provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides A_3 selective
agonists, particularly N^6-benzyladenosine-5'-uronamide
10   and related substituted compounds, particularly those
containing substituents on the benzyl and/or uronamide
groups, and xanthine riboside derivatives, as well as
pharmaceutical compositions containing such compounds.
The present invention also provides a method of
15   selectively activating an A_3 adenosine receptor in a
mammal, which method comprises acutely or chronically
administering to a mammal in need of selective activation
of its A_3 adenosine receptor a therapeutically or
prophylactically effective amount of a compound which
20   binds with the A_3 receptor so as to stimulate an A_3
receptor-dependent response.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of A_3 adenosine receptor binding
25   versus concentration of $[^{125}I]$AB-MECA (nM).

Figure 2 is a graph of total distance traveled by
mice (cm/30 min) versus dose of A_3 adenosine receptor
agonist 3-IB-MECA (μg/kg).

Figure 3 is a bar graph of total distance traveled
30   (cm/30 min) (percent of control) by mice versus type of
adenosine receptor agonist.

Figure 4 is a graph of % survivors versus days
following either 10 or 20 minute forebrain ischemia, with
(acute or chronic) and without the administration of IB-
35   MECA (0.1 mg/kg).

Figure 5 is a graph of the rate of cortical blood
flow (% change from pre-ischemia) versus time after 10

4

min cerebral ischemia (min) in the presence (acute or
chronic) and absence of the A_3 adenosine receptor agonist
IB-MECA.

Figure 6 is a bar graph of % survivors versus days
5   post-ischemia.

Figure 7 is a bar graph of % surviving neurons in
gerbil hippocampus following 10 min forebrain ischemia
versus treatment with (acute or chronic) and without 100
μg/kg IB-MECA.

10   Figure 8 is a graph of the correlation of affinity
measured in binding assays at cloned rat brain A_3
receptors (stably transfected CHO cells, using [^{125}I]APNEA
as radioligand) vs. cloned A_3 receptors from human brain
(using [^{125}I]ABA as radioligand).

15

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides for compounds which
have been found to be selective A_3 adenosine receptor
agonists, pharmaceutical compositions containing such
20   compounds, and related treatment methods and assay
methods.

The modification of adenosine at the 5'-position
and/or at the N^6-position with groups that enhance A_3
potency has been found to result in moderate A_3
25   selectivity. In particular, the 5'-methyluronamide
modification of adenosine and the N^6-benzyl group, either
alone or in combination, increases affinity in binding to
A_3 receptors relative to A_1 and A_{2a} receptors.
Optimization of substituent groups has led to the
30   development of the highly potent A_3 agonist
N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide
(IB-MECA) which is 50-fold selective for A_3 vs. either A_1
or A_2 receptors. A closely related, but less selective
radioligand, [^{125}I]AB-MECA, has been developed for
35   characterization of A_3 receptors and has been found to
have a K_d value of 3.6 nM in binding to rat A_3 receptors
in the RBL-2H3 mast cell line. While derivatives such as

N^6-benzyladenosine-5'-N-ethyluronamide have been found to be full agonists in inhibiting adenylate cyclase via rat A_3 receptors, such derivatives, while useful, are only one order of magnitude selective for rat A_3 receptors vs. either A_1 or A_{2a} receptors in binding assays.

Triple substitution of adenosine results in the further enhancement of the degree of A_3 selectivity, such that an improvement in selectivity in binding assays of three orders of magnitude or more can be achieved. By combining the two modifications at 5'- and N^6-positions, which result in moderate selectivity, with a third site of modification, particularly the 2-position, selectivity can be dramatically increased. For example, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide has been found to be the most potent and selective agent in binding assays and has been shown to be a full agonist in the inhibition of adenylate cyclase. The agonist potency was also greater than that of other agonists, indicating a parallel between binding affinities and relative potencies in this functional assay. Such agonist properties are similarly expected in another relevant functional assay, stimulation of A_3-mediated phosphoinositide metabolism.

The novel A_3 adenosine receptor is believed to be important in the regulation of CNS, cardiac, inflammatory, and reproductive functions. Activation of A_3 receptors enhances the release of inflammatory mediators from mast cells (Ramkumar et al., J. Biol. Chem., 268, 16887-16890 (1993); Ali et al., J. Biol. Chem., 265, 745-753 (1990)), lowers blood pressure (Fozard et al., Br. J. Pharmacol., 109, 3-5 (1993)), and depresses locomotor activity (Jacobson et al., FEBS Letters, 336, 57-60 (1993)). Selective agonists are believed to have therapeutic potential as cerebroprotective agents (von Lubitz et al., Drug Devel. Res., 31, 332 (Abstract 1224) (1994), and the activation of A_3 receptors is thought to be related to the

6

cardioprotective preconditioning response following
exposure to adenosine agonists. It has been discovered
that the chronic administration of an A_3 agonist provides
a cerebroprotective effect. For example, the
5   cerebroprotective effects of IB-MECA have been discovered
using an ischemic model in gerbils and NMDA-induced
seizures in mice (von Lubitz et al., Neurosci Abstr.
(1994)). Moreover, the cardioprotective potential of A_3
receptor activation, based on use of APNEA coadministered
10   with a xanthine antagonist that does not act at A_3
receptors, has been demonstrated. APNEA has been found
to be 8-fold A_1 selective, and its pharmacological use is
limited to such combination with antagonists of both A_1
and A_{2a} receptors. Clearly, the availablility of ligands
15   such as those described herein, particularly 2-chloro-N^6-
(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-
adenine, could be critical in pharmacological studies of
A_3 receptors. A highly selective A_3 ligand is expected to
be especially useful as a radioligand, since the
20   currently used high affinity ligand $[^{125}I]AB$-MECA, is not
sufficiently selective for general application in tissue
(Olah et al., Mol. Pharmacol., 45, 978-982 (1994).
        Although the selectivities of these novel A_3
agonists may vary somewhat in different species, due to
25   the unusually large species dependence in ligand affinity
at this subtype, such differences appear to be more
pronounced for antagonists than for agonists (Linden et
al., Mol. Pharmacol., 44, 524-532 (1993); Salvatore et
al., Proc. Natl. Acad. Sci. U.S.A., 90, 10365-10369
30   (1993); Brackett et al., Biochem. Pharmacol., 47, 801-814
(1994)). Thus, it should be noted that 2-chloroadenosine
is 17-fold less potent than NECA at rat A_3 receptors,
whereas at sheep A_3 receptors 2-chloroadenosine is 1.7-
fold less potent than NECA. Thus, since the most
35   selective compound in the present series, 2-chloro-N^6-(3-
iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-
adenine, contains the 2-chloro substitution, it is likely

that the selectivity will not be substantially diminshed
in other species, such as sheep and human. A high degree
of correlation in the relative affinities of adenosine
derivatives at rat vs. human A_3 receptors has been shown.

5   A high degree of selectivity exists for doubly-
substituted derivatives, such as N^6-(3-iodobenzyl)-
adenosine-5'-N-methyluronamide (IB-MECA), and triply-
substituted adenosine derivatives for A_3 receptors vs.
the NBTI-sensitive adenosine uptake site. 2-substitution

10   is well-tolerated at A_3 receptors, whether it be with a
small group (e.g., 2-chloro-N^6-(3-iodobenzyl)-adenosine)
or a large group (e.g., APEC). The potency enhancing
effects of 2-substituents appeared to follow the order:
chloro > thioether > amine. The effects of 2-

15   substitution to enhance A_3 affinity are also additive
with effects of uronamides at the 5'-position and a 3-
iodobenzyl group at the N^6-position, although the A_3
affinity-enhancing effect of a 2-chloro group do not
appear to be additive with an N^6-cyclopentyl group. The

20   combination of most favorable modifications at three
positions has led to very potent and highly selective
agonist ligands, particularly 2-chloro-N^6-(3-iodobenzyl)-
9-[5-(methylamido)-β-D-ribofuranosyl]-adenine, N^6-(3-
iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-

25   ribofuranosyl]-adenine, and N^6-(3-iodobenzyl)-2-
methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.
           A general parallel has also been found between the
structure activity relationships for adenosine A_3
agonists and xanthine ribosides, particularly in the

30   ribose moiety. The substituent effects at the 5'-
position, i.e., for uronamide derivatives, are nearly
identical. This parallel in SAR is supportive of the A_3
receptor model which features the ribose moiety of the
ligand, either adenosine or xanthine ribosides,

35   coordinated by hydrogen bonding to the same amino acid
residue, His of the seventh transmembrane helix.

8

        While the 7-ribosides have affinity for the
receptor, the 9-ribosides do not bind. For purposes of
comparing SAR, consideration may be given to the case in
which the purine rings overlap in a "flipped"
5   orientation, i.e., the xanthine N-7 occupies the same
position as the adenosine N-9, xanthine N-3 corresponds
rougly to C-6 of adenosine, etc. Thus, the position
corresponding to the critical N^6-substituent on adenosine
analogues would correspond approximately to the xanthine
10   3-substituent in the xanthine-7-ribosides, minus the α-
carbon which might occupy the space of the N^6-NH. A 3-
benzyl group, as in 3-benzyl-1-butylxanthine-7-β-D-
ribofuranoside, somewhat enhanced affinity at A_1
receptors, in parallel to the modest enhancement of
15   affinity at A_1 receptors of N^6-phenyladenosine
derivatives. Thus, at least some of the results set
forth herein to describe the present invention are
compatible with the hypothesis of a "flipped" overlap of
purines.
20   However, there are possible differences between the
two classes of nucleosides in structural determinants of
affinity on the purine ring. At the purine 8-position,
substitution is tolerated in A_3 receptor binding (8-
methoxy) for xanthine ribosides but not for adenosine
25   derivatives, as in 8-bromoadenosine. Similarly, it was
recently reported that 8-alkylamino substitution of
theophylline-7-riboside is tolerated at A_1 receptors (van
der Wenden et al., Drug Devel. Res., 31, 314 (1994)).
        Also, implications for syn vs. anti modes of binding
30   at A_1 and A_2 receptors have been derived from the affinity
of the xanthine-7-ribosides, since the xanthine ribosides
have a much stronger energetic preference for the anti-
conformation than do adenosine derivatives. Thus, based
on the absence of affinity of 8-bromoadenosine it is
35   likely that the glycosidic conformation of adenosine
binding at A_3 receptors is similar to that at A_1
receptors, i.e., in a non-syn-conformation. It is also

9

possible that the orientation is not purely *anti*, since certain 8-substitution of the xanthine ribosides is tolerated.

Xanthine-7-ribosides were initially described as
5   antagonists (van Galen et al., Nucleosides & Nucleotides, 10, 1191-1193 (1991)), but there have been some suggestions that that theophylline-7-riboside is a partial agonist at A_1 receptors (Borea et al., Int. J. Purine and Pyrimidine Res., 3, 65 (1992)), based on
10   diminished shifts in binding in the presence of guanine nucleotides. It has also been noted that many adenosine derivatives display a correlation between lower affinity and partial agonism (Borea et al., Eur. J. Pharmacol. (Mol. Pharm. Section) (1994)). It is unknown whether the
15   interconnection between intrinsic activity and affinity exists for this series of xanthine ribosides. 1,3-Dibutylxanthine-7-riboside has been found to be a partial agonist in the inhibition of adenylate cyclase via A_3 adenosine receptors, and a partial agonist may have
20   therapeutic applications, based on the case of glycine receptors (von Lubitz et al., Eur. J. Pharmacol., 219, 153 (1992)). Also, the exploration of partial agonists may lead to the development of antagonists (Jasper et al., Biochem. Pharmacol., 43, 119-130 (1992)), which are
25   lacking for A_3 receptors.

There are also common features between the structure activity relationships for dialkylxanthines binding to A_3 receptors and xanthine-7-ribosides. The 1,3-dibutyl analogues in both cases are the optimal chain length (for
30   neutral molecules). A major difference is that for the xanthines, selectivity at the rat A_3 receptor was not achieved. At A_1 receptors, the xanthines are generally more potent than the xanthine-7-ribosides, while at rat A_3 receptors the converse is true. At other species,
35   however, notably sheep and human A_3 receptors, certain xanthines, particularly those bearing negative charges, bind with high affinity.

10

For example, N-methyl 1,3-di-*n*-butylxanthine-7-β-D-
ribofuronamide has been identified as a very selective
ligand for A_3 receptors. Although it is not as potent as
the adenosine derivative, IB-MECA (5'-N-methyl-N^6-(3-
5   iodobenzyl)adenosine), it adds to the useful tools
available for studying this newly cloned subtype. The
indication that it is a partial agonist may be useful in
studying receptor regulation, which is mainly unexplored
for the A_3 receptor, and indicates that it may be useful
10   in a therapeutic capacity as, for example, a
neuroprotective agent (von Lubitz et al., <u>Neurosci.</u>
<u>Abstr.</u> (1994)) and as an antiinflammatory agent (Beaven
et al., <u>Trends Pharm. Sci.</u>, <u>15</u>, 13-14 (1994)).

15   <u>Compounds</u>

The present invention provides a compound having the
formula
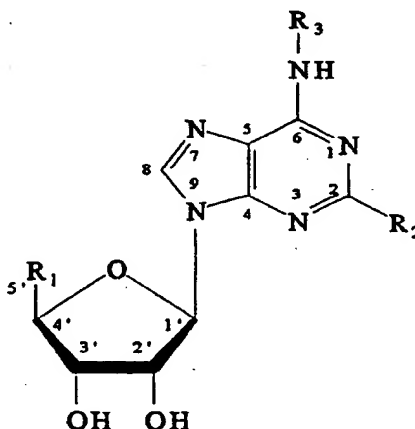
20   wherein R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a and R^b may be
the same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
C_3-C_{10} cycloalkyl, or are joined together to form a
25   heterocyclic ring containing two to five carbon atoms,
and R^c is selected from the group consisting of C_1-C_{10}
alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10}
boc-aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is selected from

the group consisting of hydrogen, halo, C_1-C_{10}
alkylethers, amino, C_1-C_{10} alkylamino, C_2-C_{10} alkenes, C_2-
C_{10} alkynes, thio, and C_1-C_{10} alkylthio, and R_3 is
selected from the group consisting of R- and S- 1-
phenylethyl, an unsubstituted benzyl group, and a
phenylethyl or benzyl group substituted in one or more
positions with a substituent selected from the group
consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo. When
R_3 is a substituted phenylethyl or benzyl group and there
is more than one substituent on the phenylethyl or benzyl
group, the substituents may be the same or different.
Moreover, these compounds may be in the form of a
suitable salt. For example, the sulfo-substituted
derivative may be a salt, such as a triethylammonium
salt.

These compounds are generally referred to as N^6-
benzyladenosine-5'-N-uronamides and derivatives thereof
and have been found to be A_3-selective adenosine receptor
agonists. Preferred compounds include those of the above
formula wherein R_1 is $R^a R^b NC(=O)$, wherein R^a and R^b may be
the same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is
selected from the group consisting of hydrogen, halo, C_1-
C_{10} alkylethers, amino, C_2-C_{10} alkenes, and C_2-C_{10} alkynes,
and R_3 is selected from the group consisting of R- and S-
1-phenylethyl, an unsubstituted benzyl group, and a
benzyl group substituted in one or more positions with a
substituent selected from the group consisting of C_1-C_{10}
alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy,
acetamido, C_1-C_{10} alkoxy, and sulfo. More preferred
compounds include those of the above formula wherein R^a
and R^b may be the same or different and are selected from
the group consisting of hydrogen and C_1-C_{10} alkyl,
particularly when R_2 is hydrogen or halo, especially
hydrogen. Additional preferred compounds are those

12

compounds wherein R^a is hydrogen and R_2 is hydrogen, particularly when R_3 is unsubstituted benzyl. More preferred compounds are such compounds wherein R^b is a C_1-C_{10} alkyl or C_3-C_{10} cycloalkyl, particularly a C_1-C_{10}

5   alkyl, and more particularly methyl. Especially preferred are those compounds where R^a is hydrogen, R^b is C_1-C_{10} alkyl or C_3-C_{10} cycloalkyl, and R_3 is R- or S- 1-phenylethyl or a benzyl substituted in one or more positions with a substituent selected from the group

10   consisting of halo, amino, acetamido, C_1-C_{10} haloalkyl, and sulfo, where the sulfo derivative is a salt, such as a triethylammonium salt. An example of an especially preferred compound is IB-MECA (as defined in Table 1 infra). In addition, those compounds in which R_2 is a C_2-

15   C_{10} alkyne of the formula R'-C≡C- where R' is a C_1-C_8 alkyl are particularly preferred. Also preferred are those compounds wherein R_2 is other than hydrogen, particularly those wherein R_2 is halo, C_1-C_{10} alkylamino, or C_1-C_{10} alkylthio, and, more preferably, when

20   additionally R^a is hydrogen, R^b is a C_1-C_{10} alkyl, and/or R_3 is a substituted benzyl. Such preferred compounds include 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine, N^6-(3-iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-ribofuranosyl]-

25   adenine, and N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.

       Compounds similar to those above, but which contain substituents in other positions, e.g., other than on the benzyl and/or uronamide groups, or in addition thereto,

30   have also been found to be A_3-selective adenosine receptor agonists. Such compounds include those of the group consisting of N^6-benzyladenosine-5'-N-alkyluronamide-N^1-oxide and N^6-benzyladenosine-5'-N-dialkyluronamide-N^1-oxide. 2-purine substitutions, such

35   as C_1-C_{10} alkylethers, amino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, and especially halo, e.g., chloro, do not adversely affect the activity of these compounds.

13

The present invention further provides a modified xanthine-7-riboside, particularly a compound having the formula
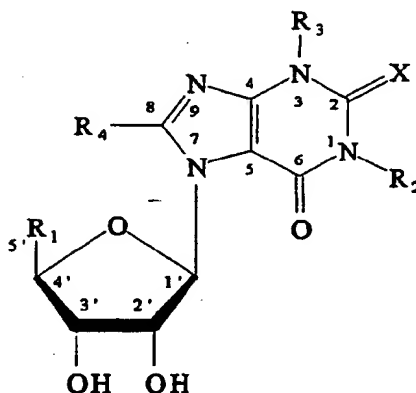
5   wherein X is O or S, R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a
       and R^b may be the same or different and are selected from
       the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-
       C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, or
10   are joined together to form a heterocyclic ring
       containing two to five carbon atoms, and R^c is selected
       from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10}
       haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
       C_3-C_{10} cycloalkyl, R_2 and R_3 may be the same or different
15   and are selected from the group consisting of C_1-C_{10}
       alkyl, R- and S- 1-phenylethyl, an unsubstituted benzyl
       group, and a phenylether or benzyl group substituted in
       one or more positions with a substituent selected from
       the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10}
20   haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and
       sulfo, and R_4 is selected from the group consisting of
       halo, benzyl, phenyl, C_3-C_{10} cycloalkyl, and C_1-C_{10}
       alkoxy, with the proviso that if X = O, R_1 is $HOCH_2$, and
       R_4 = H, then R_2 and R_3 are not C_1-C_4 alkyl, preferably not
25   C_1-C_{10} alkyl (although the compounds covered by the
       proviso are useful in the present inventive method
       described herein). Particularly preferred are those
       compounds whererin X is O, R_1 is $R^aR^bNC(=O)$, wherein R^a
       and R^b may be the same or different and are selected from
30   the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-

14

C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2
and R_3 may be the same or different and are selected from
the group consisting of C_1-C_{10} alkyl, R- and S- 1-
phenylethyl, an unsubstituted benzyl group, and a benzyl

5   group substituted in one or more positions with a
substituent selected from the group consisting of C_1-C_{10}
alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy,
acetamido, C_1-C_{10} alkoxy, and sulfo, and R_4 is selected
from the group consisting of halo, benzyl, phenyl, and

10   C_3-C_{10} cycloalkyl.

The compounds of the present invention may be used
as is or in the form of their pharmaceutically acceptable
salts and derivatives, and can be used alone or in
appropriate combination with one or more other

15   compounds/derivatives of the present invention or other
active compounds. It should be understood, however, that
the salt or derivative should not be one that renders the
compound unstable or insoluble in water or toxic at
contemplated doses. The potency of the present compounds

20   as adenosine receptor antagonists can be determined by
standard screening procedures (Bruns et al., PNAS USA,
77(9), 5547-5551 (September 1980)).

Abbreviations

25   The following abbreviations are used herein in the
course of further describing the present invention:

AB-MECA N^6-(4-amino-3-iodobenzyl)adenosine-5'-N-
            methyluronamide

ADAC N^6-[4-[[[4-[[[(2-aminoethyl)amino]carbonyl]
30   methyl]anilino]carbonyl]methyl]phenyl]adenosine

AMP   adenosine-5'-monophosphate

APNEA N^6-2-(4-aminophenyl)ethyladenosine

CPX   8-cyclopentyl-1,3-dipropylxanthine

CGS 15943   9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]
35   quinazolin-5-amine

CGS 21680   2-[4-(2-carboxyethyl)phenyl]ethylamino 5'-N-
            ethylcarboxamidoadenosine

	CHA	N^6-cyclohexyladenosine
	CP66713	4-amino-8-chloro-1-phenyl[1,2,4]-triazolo [4,3-a]quinoxaline
	CPA	N^6-cyclopentyladenosine
5	CSC	8-(3-chlorostyryl)caffeine
	CV-1808	2-(phenylamino)adenosine
	DBXR	1,3-dibutylxanthine-7-riboside
	DCCA	1-deaza-2-chloro-N^6-cyclopentyladenosine
	DMAP	4-(N,N-dimethylamino)pyridine
10	DMF	N,N-dimethylformamide
	DMSO	dimethylsulfoxide
	DPMA	N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl) ethyl]adenosine
	EDAC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
15		hydrochloride
	EHNA	erythro-9-(2-hydroxy-3-nonyl)adenine
	HMDS	1,1,1,3,3,3-hexamethyldisilazane
	IB-MECA	N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide
	IBMX	3-isobutyl-1-methylxanthine
20	IQA	imidazo[4,5-c]quinolin-4-amine
	NBTI	N^6-(4-nitrobenzyl)thioinosine
	NECA	5'-N-ethylcarboxamidoadenosine
	NMCA	5'-N-methylcarboxamidoadenosine
	NMCI	5'-N-methylcarboxamidoinosine
25	NECI	5'-N-ethylcarboxamidoinosine
	PIA	R-N^6-phenylisopropyladenosine
	R/S-PIA	N^6-[(R/S)-1-methyl-2-phenylethyl]adenosine
	SPA	N^6-(p-sulfophenyl)adenosine
	TBAF	tetrabutylammonium fluoride
30	THF	tetrahydrofuran
	TMSOTf	trimethylsilyl trifuoromethanesulfonate
	Tris	tris(hydroxymethyl)aminomethane
	XAC	8-{4-[({[(2-aminoethyl)amino]carbonyl}methyl) oxy]phenyl}-1,3-dipropylxanthine
35		

16

Compound Synthesis

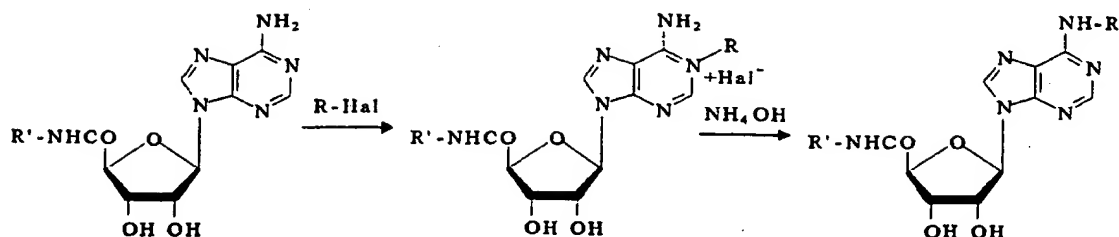The compounds of the present invention, including those useful in the present inventive compositions and methods, may be synthesized by any suitable means. The

5   compounds of the present invention are preferably synthesized by the following Reaction Scheme A.
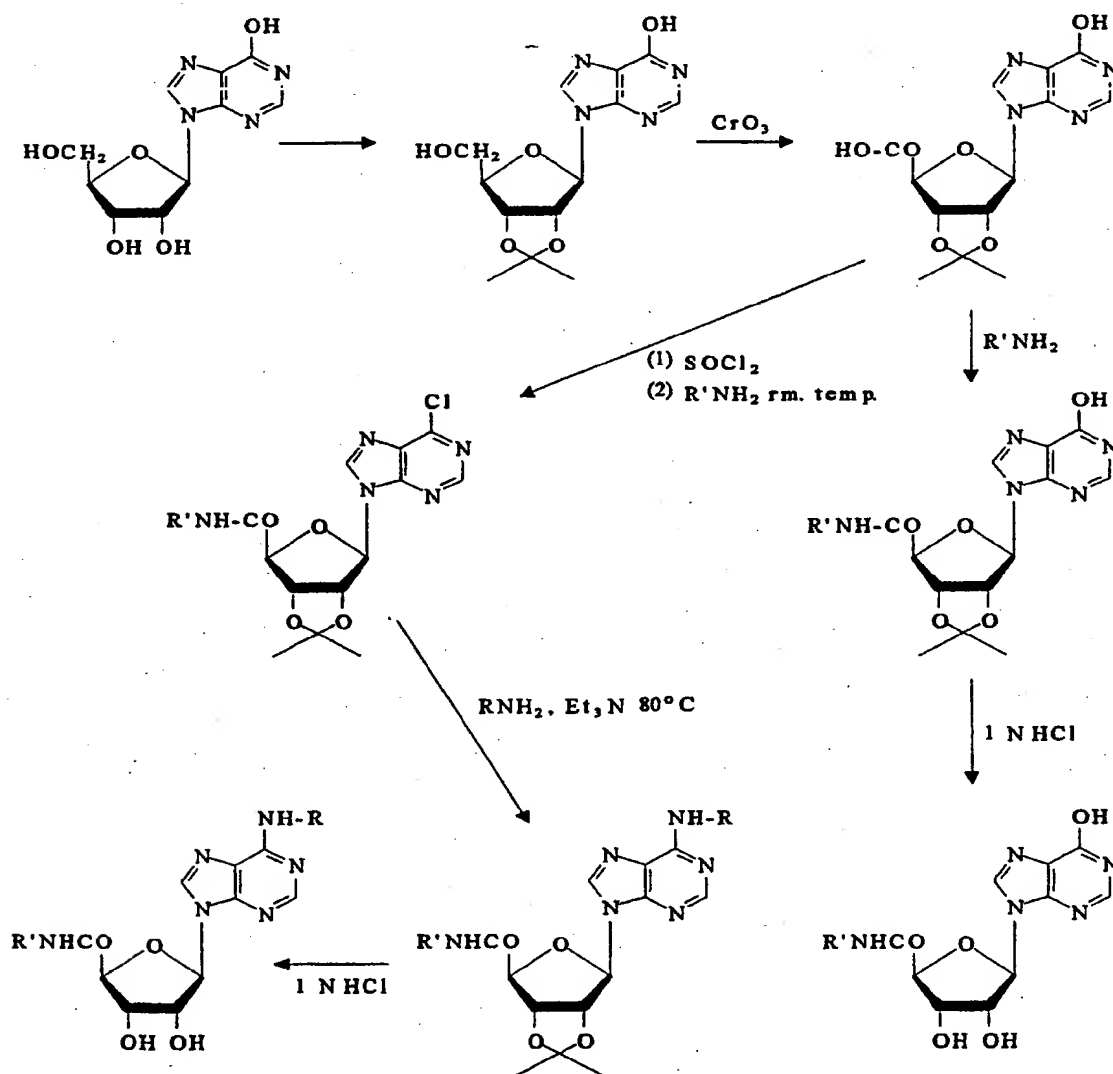

10   The compounds of the present invention may also be synthesized by a Dimroth rearrangement as set forth in the following Reaction Scheme B.

17

The details of the synthesis of the compounds of the present invention are set forth in the examples below or are reported in the literature. For example, the syntheses of the following compounds have been previously described: theophylline-7-riboside, 1,3-dipropylxanthine-7-riboside, 1,3-dibutylxanthine-7-riboside (van Galen et al., <u>Nucleosid. Nucleotid.</u>, <u>9</u>, 275-291 (1990)), imidazo[4,5-<u>c</u>]quinolin-4-amine (IQA) (van Galen et al., <u>J. Med. Chem.</u>, <u>334</u>, 1202-1206 (1991)), N^6-2-sulfoethyladenosine, N^6-4-sulfophenyladenosine, N^6-3-(4-sulfophenyl)propyladenosine, and N^6-4-(4-sulfophenyl)butyladenosine (Jacobson et al., <u>J. Med. Chem.</u>, <u>35</u>, 4143-4149 (1992)).

Various amino- (C and D) and sulfo- (E and F) substituted benzylamine intermediates for use in preparing A_3 adenosine agonists have been synthesized in accordance with the following Reaction Schemes C-F:

18

E:

5

F:
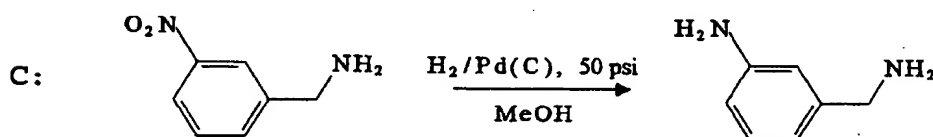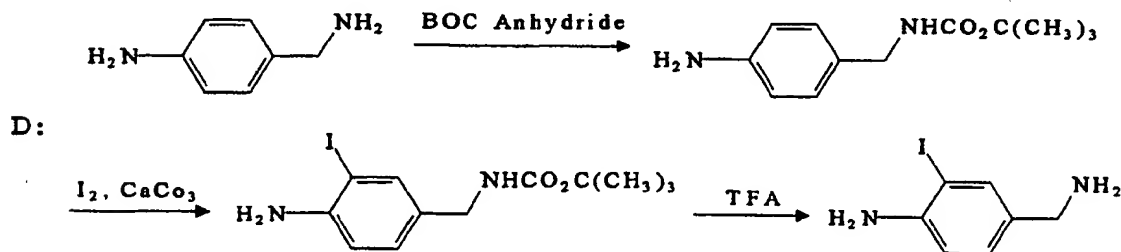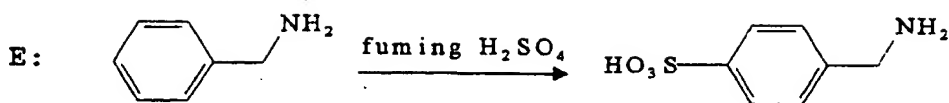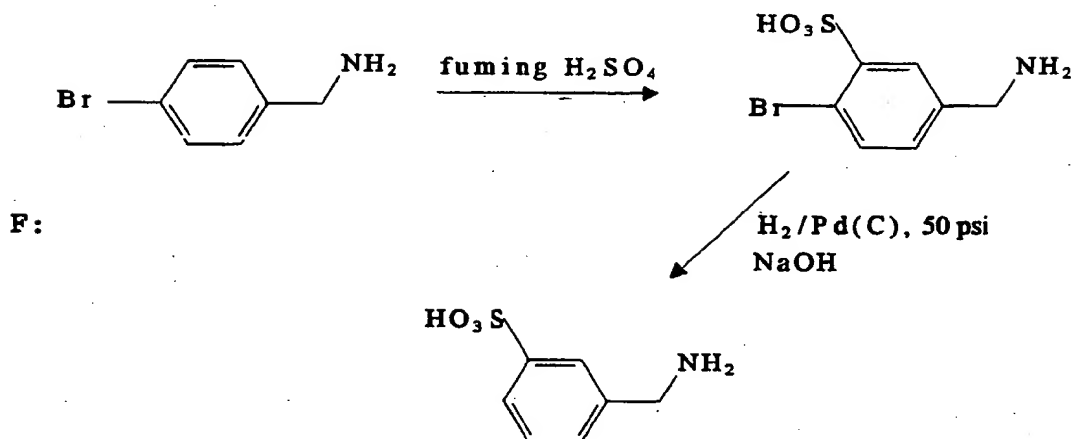
N[6]-(3-aminobenzyl)-NMCA and N[6]-(4-aminobenzyl)-NMCA,
10 respectively, have been prepared by treatment of the 6-
chloropurine intermediate (R' = CH$_3$) with 3- or 4-
aminobenzylamine. The intermediate 3-aminobenzylamine
has been prepared via catalytic reduction of the 3-nitro
derivative as shown in reaction scheme A above. The
15 nucleophilic attack of the purine ring occurs selectively
at the aryl amine. Since N[6]-(4-aminobenzyl)-NMCA has
been prepared as a precursor for radioiodination, the
expected major product of direct iodination, 3-iodo-4-
aminobenzyl-adenosine-5'-N-methyluronamide, has been
20 prepared as a standard for purification and pharmacology.
The disubstituted benzylamine intermediate has been
prepared via protection of the alkylamine as the t-
butyloxycarbonyl derivative as shown in Reaction Scheme D
above. The N[6]-(4-aminobenzyl)-NMCA has been also N-
25 acetylated at the 2',3'-isopropylidene protected stage
using acetic anhydride to yield N[6]-(3-acetamido)-NMCA
after deprotection. Two sulfo analogues, namely N[6]-(3-
sulfobenzyl)-NMCA and N[6]-(4-sulfobenzyl)-NMCA have been

prepared as shown above in Reaction Scheme F above.   The
4-sulfo derivative has been prepared from an intermediate
above prepared directly from benzylamine as shown in
reaction scheme E above.   The 3-sulfo intermediate
leading to N^6-(3-sulfobenzyl)-NMCA has been prepared via
sulfonation of 4-bromobenzylamine followed by catalytic
hydrogenation in basic medium as shown in reaction scheme
F above.

     5'-N-Boc-aminoethylamino-CA, which binds weakly but
selectively to A_3 receptors, has been synthesized as
shown in Reaction Scheme G below.
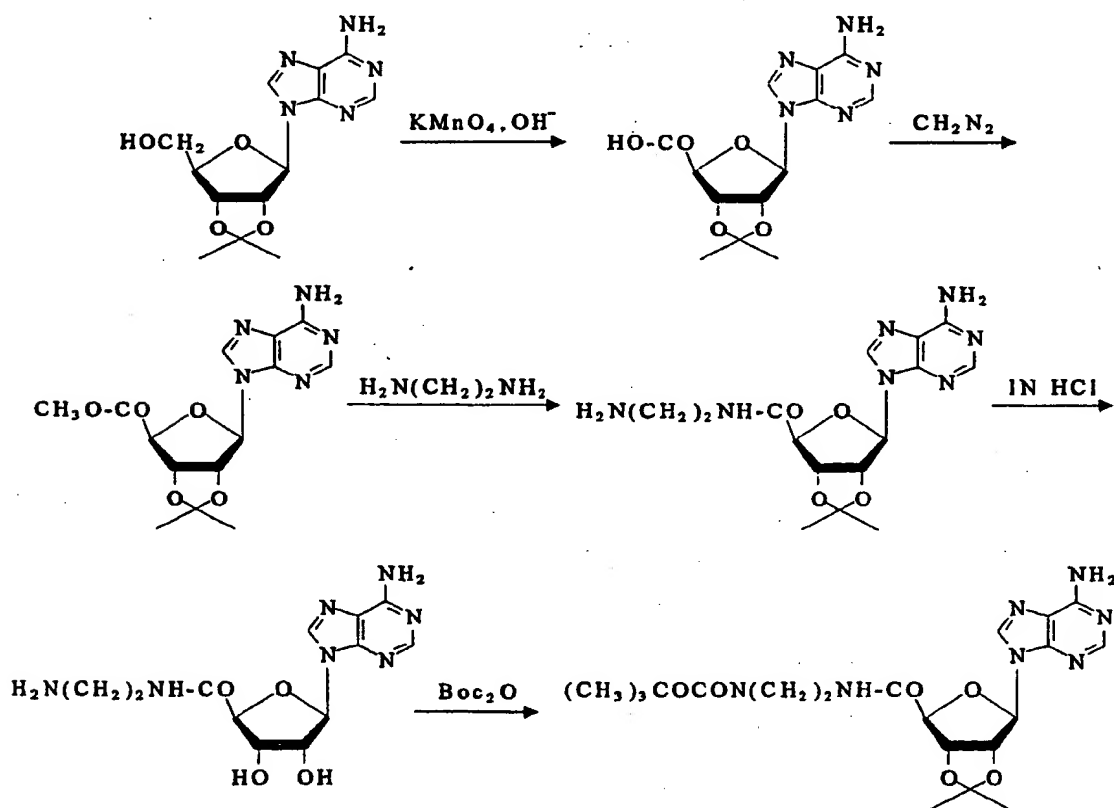
     Modified ribosides to be used in the present
inventive methods can be synthesized in accordance with
the following Reaction Schemes H and I, respectively:

20
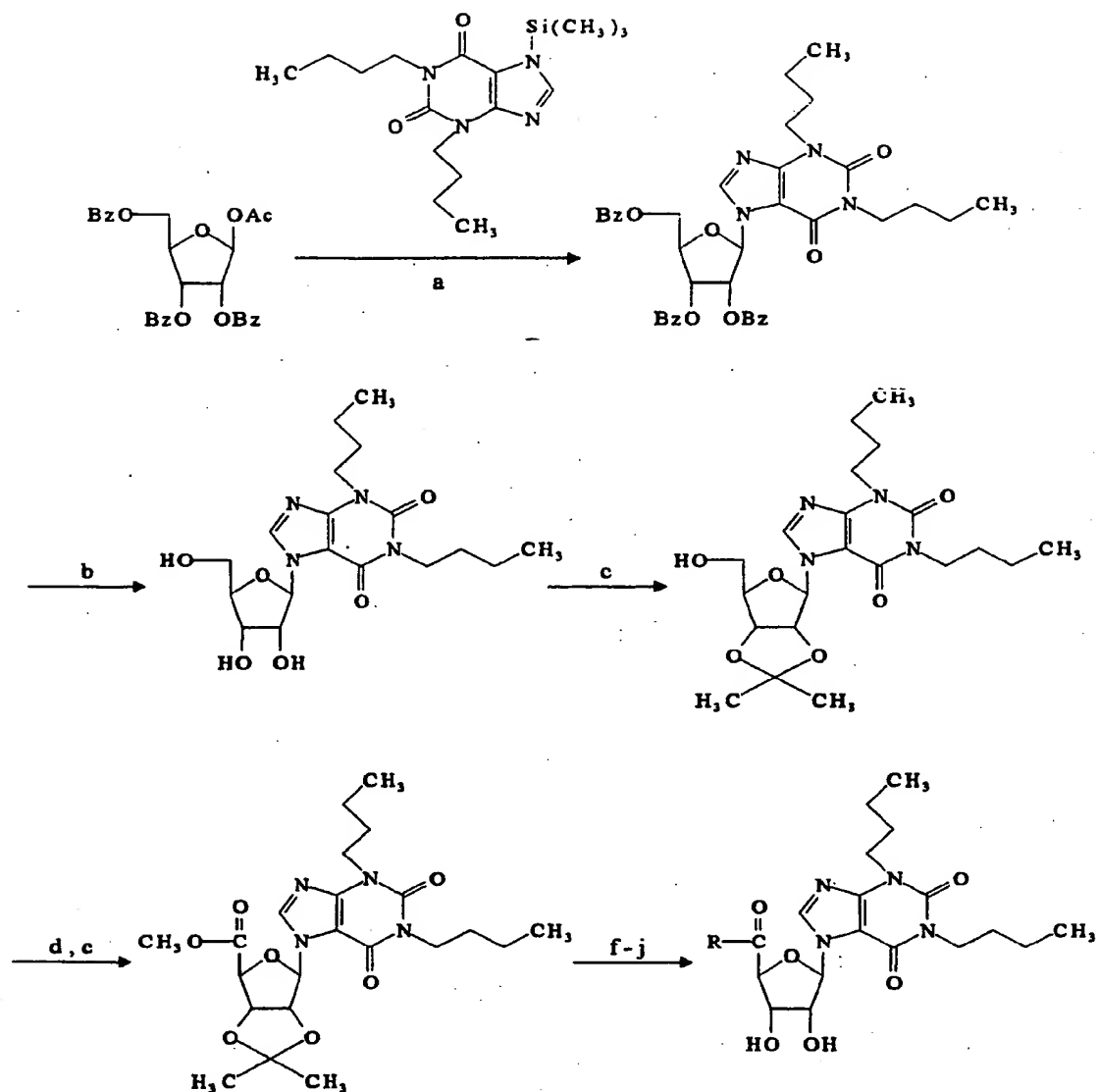
wherein the reagents and conditions are as follows:

5   (a) potassium nonaflate, $SiCl_3$, CH_3CN

     (b) NH_3/MeOH

     (c) p-TsOH, acetone

     (d) $RuCl_3$, $NaIO_4$, $CHCl_3$:CH_3CN:H_2O (2:2:3)

     (e) EDAC, DMAP, MeOH

10   (f) 88% HCO_2H

     (g) i, $MeNH_2$, MeOH, 85 °C; ii, 88% HCO_2H

     (h) i, NH_3, MeOH, 85 °C; ii, 88% HCO_2H

     (i) i, $EtNH_2$, MeOH, 85 °C; ii, 88% HCO_2H

     (j) i, Me_2NH, MeOH, 85 °C; ii, 88% HCO_2H;

or
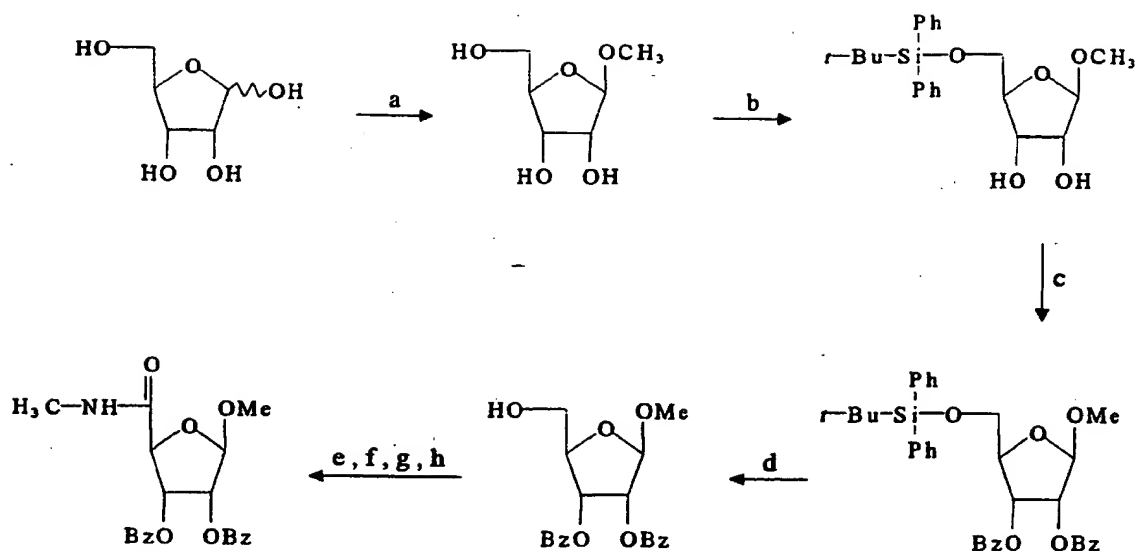
5

wherein the reagents and conditions are as follows:

(a) HCl, MeOH, room temperature, overnight

(b) TBDPSiCl, DMAP, DMF, room temperature

(c) Bz$_2$O, pyr.

10   (d) n-Bu$_4$NF, THF

(e) RuO$_2$, NaIO$_4$, CHCl$_3$:CH$_3$CN:H$_2$O (2:2:3)

(f) EDAC, DMAP, MeOH

(g) MeNH$_2$, MeOH, 75°C

(h) BzCl, pyr-CH$_2$Cl$_2$,

15 with the methyl substituent being capable of conversion
to an acetate for further reaction by techniques well
known in the art.

1,3-Dibutyl-xanthine may be synthesized according to
the procedure set forth in Jacobson et al., *J. Med.*
20 *Chem.*, *28*, 1334-1340 (1985).

The triple substitution of adenosine, i.e., at 5'-,
2-, and N[6]-positions, can be carried out by a synthetic
strategy in which a 5'-uronamide sugar moiety is
condensed with a purine moiety, such as a substituted
25 adenosine derivative. The key sugar intermediate, N-
methyl 1-O-acetyl-2,3-dibenzoyl-β-D-ribofuronamide, was
synthesized starting from methyl β-D-ribofuranoside,

which is commercially available or can be synthesized
from D-ribose (Baker et al., <u>J. Org. Chem.</u>, <u>26</u>, 4605-4609
(1961)), in 8 steps. The primary alcohol of β-D-
ribofuranoside can be selectively protected with *tert*-
5   butyldiphenylsilyl chloride (Chaudhary et al., <u>Tet.</u>
<u>Lett.</u>, <u>20</u>(2), 99-102 (1979)) to provide 1-O-methyl 5-(*t*-
butyldiphenylsilyl)-β-D-ribofuranoside, and other
alcohols can be followed with benzoyl protection to
provide 1-O-methyl 5-(*t*-butyldiphenylsilyl)-2,3-
10   dibenzoyl-β-D-ribofuranoside, the desilylation of which
with TBAF/THF will give 1-O-methyl 2,3-dibenzoyl-β-D-
ribofuranoside. The 5'-position of 1-O-methyl 2,3-
dibenzoyl-β-D-ribofuranoside can be oxidized using
ruthenium tetroxide (Singh et al., <u>Tet. Lett.</u>, <u>33</u>(17),
15   2307-2310 (1992)) to yield 1-O-methyl-2,3-dibenzoyl-β-D-
ribofuronic acid which can be purified after methylation
on a silica gel column chromatography. Methylamide at 5-
position can be introduced by nucleophilic displacement
of methyl 1-O-methyl-2,3-dibenzoyl-β-D-ribofuronate with
20   methylamine in THF and benzoyl reprotection of resulting
2,3-diol to yield the sugar intermediate N-methyl 1-O-
acetyl-2,3-dibenzoyl-β-D-ribofuronamide.
        N[6]-(3-iodobenzyl)-2-substituted adenosine
derivatives can be synthesized from the corresponding
25   adenine derivative. 2,6-Dichloropurine reacted with 3-
iodobenzylamine hydrochloride in the presence of
triethylamine in ethanol at room temperature provides N[6]-
(3-iodobenzyl)-2-chloroadenine, which can be silylated
before coupling to yield the silylated derivative. The
30   glycosidic bond is formed upon treatment of the 1'-O-
acetyl riboside derivative N-methyl 1-O-acetyl-2,3-
dibenzoyl-β-D-ribofuronamide with the aforesaid 9-
silylated adenine derivative in the presence of TMSOTf as
a Lewis acid catalyst. Condensation of N-methyl 1-O-
35   methyl-2,3-dibenzoyl-β-D-ribofuronamide with the
aforesaid 9-silylated adenine derivative produces ribose
ring-opened product. Benzoyl groups of 2-chloro-N[6]-(3-

iodobenzyl)-9-[5-(methylamido)-2,3-di-O-benzoyl-β-D-ribofuranosyl]-adenine are deprotected with NH_3/MeOH to produce 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine, which reacted with various nucleophiles such as methylamine/THF and sodium thiomethoxide/DME yield compounds N^6-(3-iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine and N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine. The benzoyl groups of 2-chloro-N^6-(3-iodobenzyl)-9-[2,3,5-tri-O-benzoyl-β-D-ribofuranosyl]-adenine can be similarly deprotected to produce the riboside derivative 2-chloro-N^6-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine.

Parallel substitutions of xanthine-7-ribosides and related analogues can be similarly carried out. The synthesis of theophylline-7-riboside and other xanthine glycosides has been described (van Galen et al., Nucleosides & Nucleotides, 9, 275-291 (1990); van Galen et al., Nucleosides & Nucleotides, 10, 1191-1193 (1991); Ozola et al., Nucleosides & Nucleotides, 12, 479-486 (1991)). Silylated xanthines can be condensed with 1-acetyl-2,3,5-tri-O-benzoyl-ribofuranoside using potassium nonaflate and trichlorosilane as Lewis acid catalyst. Unsymmetrical 1,3-disubstitution is accomplished by glycosylation of a mixture of 1-benzyl-3-butylxanthine and 3-benzyl-1-butylxanthine with 1-acetyl-2,3,5-tri-O-benzoyl-ribofuranoside followed by chromatographic separation and fractional crystalization of the isomers. As shown in Reaction Scheme J below, debenzoylation of intermediates a-g with methanolic ammonia produces the indicated xanthine ribosides, whereas intermediate h, if desired, can be converted into the corresponding methoxy derivative.

24
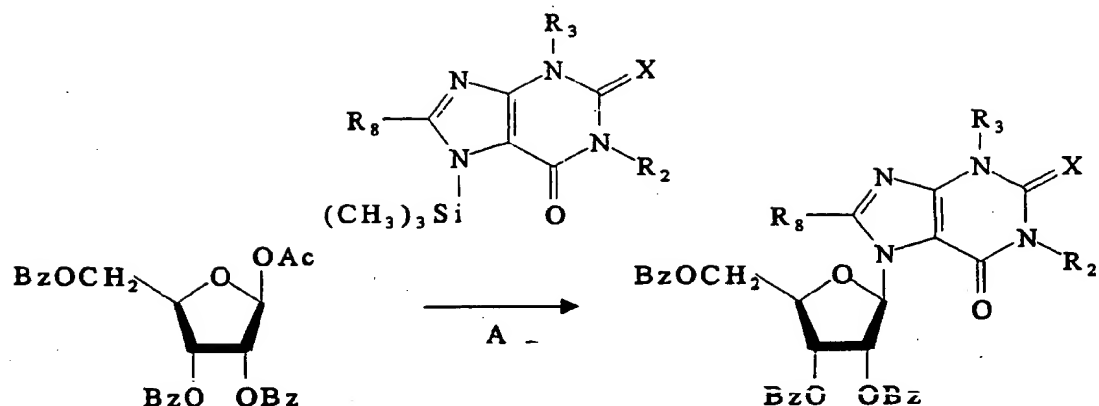
starting compound   intermediate
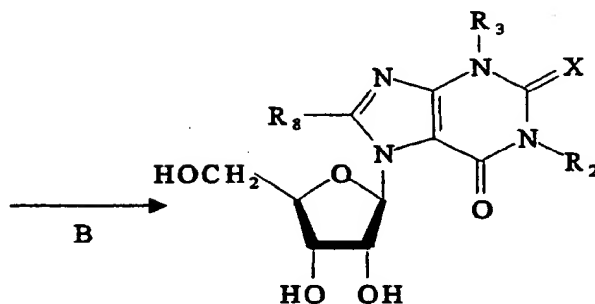
final product

	intermediate		final product
a:	$R_2=R_3=Pr$; $X=O$; $R_8=H$	→	$R_2=R_3=Pr$; $X=O$; $R_8=H$
b:	$R_2=R_3=Bu$; $X=O$; $R_8=H$	→	$R_2=R_3=Bu$; $X=O$; $R_8=H$
c:	$R_2=Bz$; $R_3=Bu$; $X=O$; $R_8=H$	→	$R_2=Bz$; $R_3=Bu$; $X=O$; $R_8=H$
d:	$R_2=Bu$; $R_3=Bz$; $X=O$; $R_8=H$	→	$R_2=Bu$; $R_3=Bz$; $X=O$; $R_8=H$
e:	$R_2=R_3=Bu$; $X=S$; $R_8=H$	→	$R_2=R_3=Bu$; $X=S$; $R_8=H$
f:	$R_2=R_3=Pent$; $X=O$; $R_8=H$	→	$R_2=R_3=Pent$; $X=O$; $R_8=H$
g:	$R_2=R_3=Hex$; $X=O$; $R_8=H$	→	$R_2=R_3=Hex$; $X=O$; $R_8=H$
h:	$R_2=R_3=Me$; $X=O$; $R_8=Cl$	→	$R_2=R_3=Me$; $X=O$; $R_8=OMe$

Reagents: A) potassium nonaflate, $SiCl_3$, CH_3CN

B) $NH_3/MeOH$

In order to synthesize 5'-modified xanthine
nucleosides, 2'- and 3'-hydroxyl groups were selectively
protected by isopropylidenation. Oxidation of the 5'-
hydroxyl group was accomplished under mild conditions
using ruthenium chloride, in a procedure modified from
Singh et al., Tetrahedron Letters, 33, 2307-2310 (1992).

When 0.1 equivalent of ruthenium chloride is used, as described in the literature, deglycosylation is observed, and the reaction cannot be controlled. Reduction of the number of equivalents to 0.01 resulted in an increase in reaction time and a decrease in deglycosylation. Esterification of the 5'-carboxylic acid was accomplished by a modification of the Hassner procedure. The methyl ester can be purified by silica gel column chromatography. The replacement of the methyl ester group with several nucleophiles, such as methylamine, ammonia, dimethylamine, and ethylamine, followed by deisopropylidenation, produces 5'-modified xanthine ribosides, such as those of Examples 65-67 and 69-71. For the preparation of 8-substituted xanthine ribosides bromination using bromine, or N-bromosuccinimide (NBS), can be utilized on 1,3-dibutyl-7-[2,3-isopropylidene-5-(methylamido)-β-D-ribofuranosyl]-xanthine, 1,3-dipentylxanthine-riboside, or 2',3',5'-triacetyl-1,3-dipentyl-xanthine-riboside. Direct condensations of 8-substituted-1,3-dibutylxanthine with the riboside sugar of Reaction Scheme J above (i.e., the starting compound) under similar condensation condition can also be attempted. However, substitutions of the 8-position on xanthine are not entirely successful, probably due to steric hindrance of 1-butyl or 1-pentyl group. Changing the substituents at the 1- and 3-positions of xanthine from butyl or pentyl to methyl group allowed the condensation to work. Thus the aforesaid riboside sugar was condensed with 8-chloro-theophylline under similar condensation conditions to give intermediate h, which was converted to the 8-methoxy derivative 8-methoxy-theophylline-7-β-D-ribofuranoside in NH3/MeOH.

Pharmaceutical Compositions

The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount, e.g., a therapeutically

26

effective amount, including a prophylactically effective
amount, of one or more of the aforesaid compounds.

The carrier can be any of those conventionally used
and is limited only by chemico-physical considerations,
5   such as solubility and lack of reactivity with the
compound, and by the route of administration. It will be
appreciated by one of skill in the art that, in addition
to the following described pharmaceutical compositions,
the compounds of the present invention can be formulated
10 as inclusion complexes, such as cyclodextrin inclusion
complexes, or liposomes.

Examples of pharmaceutically acceptable acid
addition salts for use in the present inventive
pharmaceutical composition include those derived from
15 mineral acids, such as hydrochloric, hydrobromic,
phosphoric, metaphosphoric, nitric and sulphuric acids,
and organic acids, such as tartaric, acetic, citric,
malic, lactic, fumaric, benzoic, glycolic, gluconic,
succinic, and arylsulphonic, for example p-
20 toluenesulphonic, acids.

The pharmaceutically acceptable carriers described
herein, for example, vehicles, adjuvants, excipients, or
diluents, are well-known to those who are skilled in the
art and are readily available to the public. It is
25 preferred that the pharmaceutically acceptable carrier be
one which is chemically inert to the active compounds and
one which has no detrimental side effects or toxicity
under the conditions of use.

The choice of carrier will be determined in part by
30 the particular active agent, as well as by the particular
method used to administer the composition. Accordingly,
there is a wide variety of suitable formulations of the
pharmaceutical composition of the present invention. The
following formulations for oral, aerosol, parenteral,
35 subcutaneous, intravenous, intramuscular,
interperitoneal, rectal, and vaginal administration are
merely exemplary and are in no way limiting.

　　　Formulations suitable for oral administration can
consist of (a) liquid solutions, such as an effective
amount of the compound dissolved in diluents, such as
water, saline, or orange juice; (b) capsules, sachets,
5　tablets, lozenges, and troches, each containing a
predetermined amount of the active ingredient, as solids
or granules; (c) powders; (d) suspensions in an
appropriate liquid; and (e) suitable emulsions. Liquid
formulations may include diluents, such as water and
10　alcohols, for example, ethanol, benzyl alcohol, and the
polyethylene alcohols, either with or without the
addition of a pharmaceutically acceptable surfactant,
suspending agent, or emulsifying agent. Capsule forms
can be of the ordinary hard- or soft-shelled gelatin type
15　containing, for example, surfactants, lubricants, and
inert fillers, such as lactose, sucrose, calcium
phosphate, and corn starch. Tablet forms can include one
or more of lactose, sucrose, mannitol, corn starch,
potato starch, alginic acid, microcrystalline cellulose,
20　acacia, gelatin, guar gum, colloidal silicon dioxide,
croscarmellose sodium, talc, magnesium stearate, calcium
stearate, zinc stearate, stearic acid, and other
excipients, colorants, diluents, buffering agents,
disintegrating agents, moistening agents, preservatives,
25　flavoring agents, and pharmacologically compatible
carriers. Lozenge forms can comprise the active
ingredient in a flavor, usually sucrose and acacia or
tragacanth, as well as pastilles comprising the active
ingredient in an inert base, such as gelatin and
30　glycerin, or sucrose and acacia, emulsions, gels, and the
like containing, in addition to the active ingredient,
such carriers as are known in the art.
　　　The compounds of the present invention, alone or in
combination with other suitable components, can be made
35　into aerosol formulations to be administered via
inhalation. These aerosol formulations can be placed
into pressurized acceptable propellants, such as

dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

5   Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended

10   recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound can be administered in a physiologically acceptable diluent in a pharmaceutical

15   carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, glycerol ketals,

20   such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or

25   a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

       Oils, which can be used in parenteral formulations

30   include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid.

35   Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in parenteral formulations include fatty alkali metal,

ammonium, and triethanolamine salts, and suitable
detergents include (a) cationic detergents such as, for
example, dimethyl dialkyl ammonium halides, and alkyl
pyridinium halides, (b) anionic detergents such as, for
5   example, alkyl, aryl, and olefin sulfonates, alkyl,
olefin, ether, and monoglyceride sulfates, and
sulfosuccinates, (c) nonionic detergents such as, for
example, fatty amine oxides, fatty acid alkanolamides,
and polyoxyethylenepolypropylene copolymers, (d)
10   amphoteric detergents such as, for example, alkyl-β-
aminopropionates, and 2-alkyl-imidazoline quaternary
ammonium salts, and (3) mixtures thereof.

     The parenteral formulations will typically contain
from about 0.5 to about 25% by weight of the active
15   ingredient in solution. Suitable preservatives and
buffers can be used in such formulations. In order to
minimize or eliminate irritation at the site of
injection, such compositions may contain one or more
nonionic surfactants having a hydrophile-lipophile
20   balance (HLB) of from about 12 to about 17. The quantity
of surfactant in such formulations ranges from about 5 to
about 15% by weight. Suitable surfactants include
polyethylene sorbitan fatty acid esters, such as sorbitan
monooleate and the high molecular weight adducts of
25   ethylene oxide with a hydrophobic base, formed by the
condensation of propylene oxide with propylene glycol.
The parenteral formulations can be presented in unit-dose
or multi-dose sealed containers, such as ampules and
vials, and can be stored in a freeze-dried (lyophilized)
30   condition requiring only the addition of the sterile
liquid carrier, for example, water, for injections,
immediately prior to use. Extemporaneous injection
solutions and suspensions can be prepared from sterile
powders, granules, and tablets of the kind previously
35   described.

     The compounds of the present invention may be made
into injectable formulations. The requirements for

30

effective pharmaceutical carriers for injectable
compositions are well known to those of ordinary skill in
the art. See Pharmaceutics and Pharmacy Practice, J.B.
Lippincott Co., Philadelphia, PA, Banker and Chalmers,
5   eds., pages 238-250 (1982), and ASHP Handbook on
Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986).
        Additionally, the compounds of the present invention
may be made into suppositories by mixing with a variety
of bases, such as emulsifying bases or water-soluble
10   bases. Formulations suitable for vaginal administration
may be presented as pessaries, tampons, creams, gels,
pastes, foams, or spray formulas containing, in addition
to the active ingredient, such carriers as are known in
the art to be appropriate.
15

Methods of Use
        In addition, the present invention provides a method
of selectively activating A_3 adenosine receptors in a
mammal, which method comprises acutely or chronically
20   administering to a mammal in need of selective activation
of its A_3 adenosine receptors a therapeutically effective
amount, including a prophylactically effective amount, of
a compound which binds with the A_3 receptor so as to
stimulate an A_3 receptor-dependent response.
25   Preferred compounds for use in the present inventive
method include those compounds described above and
compounds having the formula

wherein R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and

5   C_3-C_{10} cycloalkyl, or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is selected from the group consisting

10   of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_1-C_{10} alkylamino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, thio, and C_1-C_{10} alkylthio, and R_3 is selected from the group consisting of R- and S- 1-phenylethyl, an unsubstituted benzyl group, and a phenylethyl or benzyl group
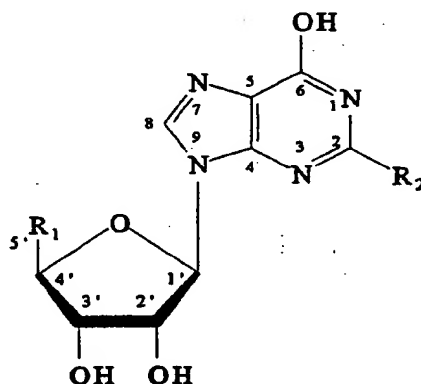
15   substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo. More preferred compounds include those compounds wherein R_1 is $R^aR^bNC(=O)$, wherein R^a and

20   R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_2-C_{10} alkenes, and C_2-C_{10} alkynes,

25   and R_3 is selected from the group consisting of R- and S-1-phenylethyl, an unsubstituted benzyl group, and a benzyl group substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy,

30   acetamido, C_1-C_{10} alkoxy, and sulfo. Additional preferred compounds include those compounds wherein R^a is hydrogen and R^b is selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and C_3-C_{10} cycloalkyl, and even

35   more preferably R^a is hydrogen and R^b is a C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_1-C_{10} aminoalkyl, or C_1-C_{10} boc-aminoalkyl. Especially preferred are those compounds

32

wherein R^a is hydrogen and R^b is a C_1-C_{10} alkyl, such as
methyl or ethyl, a C_3-C_{10} cycloalkyl, such as cyclopropyl,
a C_1-C_{10} aminoalkyl, such as aminoethyl, and a C_1-C_{10} boc-
aminoalkyl, such as boc-aminoethyl. Particularly
5   preferred compounds include 5'-N-aminoethylamino-
carboxamidoadenosine and 5'-N-boc-aminoethylamino-
carboxamidoadenosine.

Also useful in the context of the present inventive
method are those compounds having the formula

10

wherein R_1 is $R^a R^b NC(=O)$ or HOR^c, wherein R^a and R^b may be
the same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
15   haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
C_3-C_{10} cycloalkyl, or are joined together to form a
heterocyclic ring containing two to five carbon atoms,
and R^c is selected from the group consisting of C_1-C_{10}
alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-
20   C_{10} cycloalkyl, and R_2 is selected from the group
consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino,
C_1-C_{10} alkylamino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, thio,
and C_1-C_{10} alkylthio. More preferred are those compounds
wherein R_1 is $R^a R^b NC(=O)$, wherein R^a and R^b may be the
25   same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
C_3-C_{10} cycloalkyl, and R_2 is selected from the group
consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino,
30   C_2-C_{10} alkenes, and C_2-C_{10} alkynes. Most preferred are

33

those compounds wherein R^a is hydrogen and R_2 is hydrogen, particularly when R^b is hydrogen, a C_1-C_{10} alkyl, such as methyl or ethyl, a C_3-C_{10} cycloalkyl, such as cyclopropyl, a C_1-C_{10} aminoalkyl, a C_1-C_{10} aminoethyl, or a C_1-C_{10} boc-

5   aminoalkyl, such as boc-aminoethyl. Such preferred compounds include 5'-N-aminoethylamino-carboxamidoadenosine and 5'-N-boc-aminoethylamino-carboxamidoadenosine.

In addition to the modified xanthine-7-ribosides

10 disclosed above, other preferred modified xanthine-7-ribosides can also be used in the present inventive method. Such a modified xanthine-7-riboside is 1,3-R_1R_2-xanthine-7-riboside, wherein R_1 and R_2 may be the same or different and are selected from the group consisting of

15 hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl, and C_3-C_{10} cycloalkyl, or, less preferably, are joined together to form a heterocyclic ring containing two to five carbon atoms. Especially preferred such compounds include 1,3-dialkylxanthine-7-

20 ribosides, particularly 1,3-dibutylxanthine-7-riboside. Another modified xanthine-7-riboside which can be used in the present inventive method is 5'-R_3-1,3-R_1R_2-xanthine-7-riboside, wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen,

25 C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl, and C_3-C_{10} cycloalkyl, or, less preferably, are joined together to form a heterocyclic ring containing two to five carbon atoms, and R_3 is selected from the group consisting of C_1-C_{10} alkoxycarbonyl and

30 aminocarbonyl, wherein the amino group is substituted at one or more positions with a C_1-C_{10} alkyl. Especially preferred compounds of this latter group include 5'-R_3-1,3-dibutylxanthine-7-riboside, particularly 5'-methylaminocarbonyl-1,3-dibutylxanthine-7-riboside.

35 The compounds of the present invention can be utilized _in vitro_ for scientific and research purposes. For example, the present invention provides an assay,

34

which comprises providing one of the aforesaid compounds,
which has been suitably labeled, contacting a sample with
the labeled compound under conditions sufficient to
effect binding between the labeled compound and a
5   component of the sample, and determining whether any
binding occurred. The compounds of the present
invention, such as 5'-N-methyl-N^6-(3-halobenzyl)
adenosine 5'-N-methyl uronamides and iodinatable aryl
amines (e.g., compounds 44 and 50 in Table 1, infra), may
10   be used to probe A_3 adenosine receptors in order to
isolate or characterize the receptors, including their
physiological role, distribution, and regulation. In
particular, the compounds of the present invention may be
labeled, e.g., radioiodinated, for photoaffinity
15   crosslinking to the receptor protein. The crosslinking
to the receptor can be carried out with the photoaffinity
crosslinking reagent SANPAH (N-succinimidyl-6(4'-azido-
2'-nitro-phenyl-amino)hexanoate) or by conversion of the
aryl amino group to an azide, followed by photolysis in
20   the presence of the receptor. Alternatively, a
chemically reactive bifunctional reagent, such as p-
phenylene diisothiocyanate, can be coupled to the amine
congener, in a manner that leaves one electrophilic group
unreacted. Another type of label or reporter group, a
25   fluorescent dye, such as fluorescein isothiocyanate, may
be coupled to an amine congener to provide an affinity
probe. These probes obviate the need for radioactive
ligands for receptor characterization in studies
utilizing membrane homogenates and tissue slices. A
30   suitable reaction scheme for labeling a compound of the
present invention, wherein R' = methyl is preferred, is
set forth below as Reaction Scheme K.

35
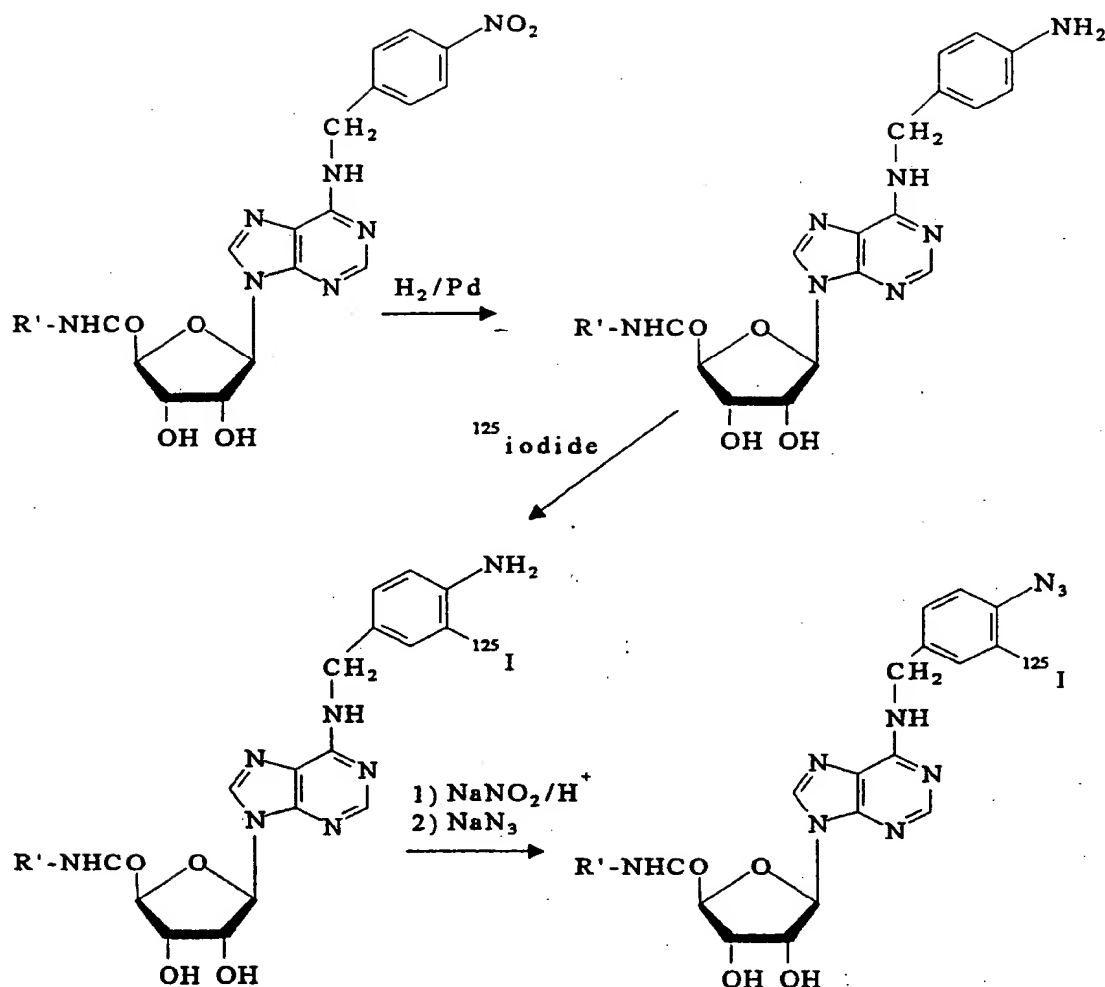
A compound of the present invention can be also linked to
an amine functionalized agarose matrix for affinity
chromatography involving the A_3-receptor.

The method of the present invention has particular
usefulness in _in vivo_ applications. For example, A_3
adenosine receptor agonists can be used in the treatment
of any disease state or condition involving the release
of inositol-1,4,5-triphosphate (IP3), diacylglycerol
(DAG), and free radicals and subsequent arachidonic acid
cascades. Thus, high blood pressure, locomotor
hyperactivity, hypertension, acute hypoxia, depression,
and infertility can be treated in accordance with the
present inventive method, wherein one of the above-
described compounds is acutely administered, e.g., within

about a few minutes to about an hour of the onset or
realization of symptoms. The method also has utility in
the treatment of chronic disease states and conditions,
in particular those conditions and disease states wherein
chronic prophylactic or therapeutic administration of one
of the above-described compounds will prevent the onset
of symptoms or will reduce recovery time. Examples of
disease states and conditions that may be chronically
treated in accordance with the present inventive method
include inflammatory disorders, such as vascular
inflammation and arthritis, allergies, asthma, wound
healing, stroke, cardiac failure, acute spinal cord
injury, acute head injury or trauma, seizure, neonatal
hypoxia (cerebral palsy; prophylactic treatment involves
chronic exposure through placental circulation), chronic
hypoxia due to arteriovenous malformations and occlusive
cerebral artery disease, severe neurological disorders
related to excitotoxicity, Parkinson's disease,
Huntington's chorea, and other diseases of the central
nervous system (CNS), cardiac disease, kidney disease,
and contraception.

Moreover, the above compounds have been found to
increase basal or systemic blood pressure, and thus the
chronic administration of these compounds can be used to
treat malignant hypotension. For example, the
administration of IB-MECA results in a signficant
increase (e.g., about 10-30%) in basal or systemic blood
pressure (e.g., from about 70 mm Hg to about 90 mm Hg).

Such compounds have also been found to be
significant cerebral protectants. As such, the above
compounds can be used to treat and/or protect against a
variety of disorders, including, for example, seizures,
transient ischemic shock, strokes, focal ischemia
originating from thrombus or cerebral hemorrhage, global
ischemia originating from cardiac arrest, trauma,
neonatal palsy, hypovolemic shock, and hyperglycemia and
associated neuropathies. The above compounds,

37

particularly, for example, IB-MECA, have also been found
to have procognitive effects and, therefore, can be used
in the treatment of disorders wherein the elicitation of
such an effect would prove useful, such as in the
5   treatment of Alzheimer's disease and other dementing and
cognitive disorders.

Although any of the above compounds may be
administered chronically, the above-described modified
xanthine-7-ribosides are preferred for chronic dosage
10   regimens. Modified xanthine-7-ribosides are preferred in
the present inventive method necessitating chronic
administration because their use under such conditions is
generally not accompanied by the adverse side-effects
typically encountered with other compounds. For example,
15   1,3-dibutyl-xanthine-riboside had a shallow dose response
curve in inhibition of adenylate cyclase in CHO cells
stably transfected with rat A_3 adenosine receptor cDNA
(in comparison with N^6-benzyl-NECA or N^6-cyclohexyl-NECA).
Therefore, these derivatives are partial agonists and, as
20   such, produce desensitization of the receptor without the
side-effects of acute administration. Cf. von Lubitz et
al., Eur. J. Pharmacol., 219, 153-158 (1993).

The present inventive method includes the
administration to an animal, such as a mammal,
25   particularly a human, in need of the desired A_3 receptor-
dependent response of an effective amount, e.g., a
therapeutically effective amount, of one or more of the
aforementioned present inventive compounds or
pharmaceutically acceptable salts or derivatives thereof,
30   alone or in combination with one or more other
pharmaceutically active compounds.

Some of the compounds of the present invention can
be utilized as functionalized congeners for coupling to
other molecules, such as amines and peptides. The use of
35   such congeners provide for increased potency, prolonged
duration of action, specificity of action, and prodrugs.
Water solubility is also enhanced, which allows for

38

reduction, if not complete elimination, of undesirable binding to plasma proteins and partition into lipids. Accordingly, improved pharmacokinetics can be realized.

One skilled in the art will appreciate that suitable
5   methods of administering a compound of the present invention to an animal are available, and, although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective reaction than another route.
10 Accordingly, the above-described methods are merely exemplary and are in no way limiting.

The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a prophylactic or therapeutic
15 response in the animal over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors including the strength of the particular compound employed, the age, species, condition, and body weight of the animal, as well as the
20 severity/stage of the disease or condition. The size of the dose will also be determined by the route, timing and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound and
25 the desired physiological effect. It will be appreciated by one of skill in the art that various conditions or disease states, in particular chronic conditions or disease states, may require prolonged treatment involving multiple administrations.
30 Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is
35 increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in

39

portions during the day if desired. In proper doses and
with suitable administration of certain compounds, the
present invention provides for a wide range of selective
A_3 receptor-dependent responses. Exemplary dosages range
5   from about 0.1 to about 100 mg/kg body weight of the
animal being treated/day. Therapeutically effective
dosages range from about 0.01 to about 10 mg/kg body
weight/day.

10   Examples

          The following examples further illustrate the
present invention and, of course, should not be construed
as in any way limiting its scope. In the examples,
unless otherwise noted, compounds were characterized and
15   resonances assigned by 300 MHz proton nuclear magnetic
resonance mass spectroscopy using a Varian GEMINI-300 FT-
NMR spectrometer. Also, unless noted otherwise, chemical
shifts are expressed as ppm downfield from
tetramethylsilane. Synthetic intermediates were
20   characterized by chemical ionization mass spectrometry
(NH_3) and adenosine derivatives by fast atom bombardment
mass spectrometry (positive ions in a noba or m-bullet
matrix) on a JEOL SX102 mass spectrometer. In the EI
mode accurate mass was determined using a VG7070F mass
25   spectrometer. All adenosine derivatives were judged to
be homogeneous using thin layer chromatography (silica,
0.25 mm, glass-backed, Alltech Assoc., Deerfield, IL;
analytical TLC plates and silica gel (230-400 mesh), VRW,
Bridgeport, NJ) following final purification.

30

Example 1

          This example describes the synthesis of N^6-
benzyladenosine-N^1-oxide.

          N^6-Benzyladenosine (25 mg, 70 μmol) and m-
35   chloroperbenzoic acid (38 mg, 220 μmol) were dissolved in
1 ml acetic acid. The resulting solution was stirred at
room temperature for 2 days. The solvent was evaporated

40

under a stream of nitrogen, and the residue was dissolved
in a minimum of methanol and chromatographed on a silica
plate (250 μ) using acetonitrile:water, 4:1 (v/v). The UV
absorbing band at R_f = 0.53 was extracted with methanol
5   to provide 7.3 mg (28% yield) of N^6-benzyladenosine-N^1-
oxide. Mass and ^{1}H-NMR spectra were consistent with the
assigned structure.

Example 2

10   This example describes the synthesis of adenosine-
5'-N-ethyluronamide-N^1-oxide.   Adenosine-5'-N-
ethyluronamide-N^1-oxide was synthesized by a method
similar to that of Example 1. Following
recrystallization from hot methanol/ether, pure
15   adenosine-5'-N-ethyluronamide-N^1-oxide was obtained in
28% yield. Mass (EI, peaks at 324 (m), 308) and ^{1}H-NMR
spectra were consistent with the assigned structure.

Example 3

20   This example describes the synthesis of N^6-
benzyladenosine-5'-N-ethyluronamide.
To a solution of 5'-N-ethylcarboxamidoadenosine
(NECA, 50 mg, 0.162 mmol) in dimethylformamide (DMF, 1
ml) was added benzyl bromide (56 ml, 0.47 mmol). The
25   resulting solution was stirred for two days at 40 °C
while protected from moisture. DMF was removed under
vacuum resulting in a syrup that crystallized when
acetone and ether were added. The solvent was removed by
decantation, and the solid was dried and dissolved in
30   methanol (2 ml). K_2CO_3 (10 mg) was added and warmed under
reflux overnight. The reaction mixture was cooled,
filtered, and evaporated. The product was purified by
preparative thin layer chromatography (TLC) ($CHCl_3$:MeOH
13:2) in 42% yield. Mp: 170-173 °C. ^{1}H NMR (in DMSO-d_6): δ
35   1.06 (t, J=7 Hz,3H, CH_3), 3.20 (m, 2H, CH_2), 4.13 (t, J=4
Hz, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.62 (m, 1H, H-2'),
4.71 (br. s, 2H, N^6-CH_2Ph), 5.53 (d, J=7 Hz, 1H, OH-2'),

41

5.73 (d, J=4 Hz, 1H, OH-3'), 5.96 (d, J=8 Hz, 1H, H-1'),
7.30 (m, 5H, Phenyl), 8.25 (s, 1H, H-2), 8.42 (s, 1H, H-8), 8.55 (br. s, 1H, N^6H-CH$_2$Ph), 8.86 (t, J=5 Hz, 1H, NH-Et). Mass Spectrum (CI-NH$_3$): m/e 399 (MH$^+$, base).

5

Example 4

This example describes the synthesis of inosine-5'-N-ethyluronamide (NECI).   2',3'-O-Isopropylideneinosine-5'-uronic acid (20 mg, 62 μmol) (Olsson et al., J. Med. Chem., 29, 1683-1689 (1986)), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (25 mg, 130 μmol), and N-hydroxysuccinimide (13 mg, 112 μmol) were dissolved in a minimum volume of DMF. Ethylamine (70% in water, 7 μl) was added and, after 1 hour of stirring, was cooled to 0 °C and precipitated with water to yield 14 mg (65% yield). The product (10 mg, 29 μmol) was heated in 1 N HCl for 2 hours at 60°C. After cooling and neutralizing with NaHCO$_3$, the product was purified twice using reverse phase SepPak cartridges, with water as eluant. Lyophilization of the fraction afforded 6.95 mg (78% yield) of an amorphous solid. Mp : 168 °C (d). ^{1}H NMR (in DMSO-d$_6$): δ 1.03 (t, J=7 Hz,3H, CH$_3$), 3.17 (m, 2H, CH$_2$), 4.15 (br s, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.54 (m, 1H, H-2'),5.61 (br s, 1H, OH), 5.68 (br. s, 1H, OH), 5.96 (d, J=7 Hz, 1H, H-1'), 8.08 (s, 1H, H-2), 8.39 (s, 1H, H-8). Mass spectrum (CI-NH$_3$): m/e 310 (MH$^+$, base).

Example 5

This example describes the synthesis of N^6-(4-nitrobenzyl)adenosine-5'-N-ethyluronamide.

To a solution of NECA (50 mg, 0.162 mmol) in DMF (0.5 ml) was added 4-nitrobenzyl bromide (53 mg, 0.245 mmol), and the solution was stirred for two days at 40°C. DMF was removed under vacuum giving a syrup that crystallized when acetone and ether were added. The solvent was removed using a Pasteur pipette.   Methanol

42

(1.0 ml) and concentrated NH$_4$OH (2.0 ml) were added, and
the mixture was warmed in a closed vessel at 90 °C for 45
min. The solvent was evaporated, and the product was
purified by preparative TLC (silica gel, ethyl acetate:

5   isopropanol:water, 4:1:2) to yield 7 mg of the pure
product (R$_f$ 0.77, 20% yield). Mp: 196°C.

   ^{1}H NMR (in DMSO-d$_6$): δ 1.06 (t, J=7 Hz,3H, CH$_3$), 3.20
(m, 2H, CH$_2$), 4.13 (br. s, 1H, H-3'), 4.30 (s, 1H, H-4'),
4.60 (m, 1H, H-2'), 4.82 (br. s, 2H, N^6-CH$_2$Ph), 5.55 (d,

10   J=6 Hz, 1H, OH-2'), 5.73 (d, J=4 Hz, 1H, OH-3'), 5.97 (d,
J=7 Hz, 1H, H-1'), 7.58 (d, 2H, J=9 Hz, arom), 8.17 (d,
2H, J=9 Hz, arom), 8.25 (s, 1H, H-2), 8.46 (s, 1H, H-8),
8.70 (br. s, 1H, N^{6}H-CH$_2$Ph), 8.81 (t, J=5 Hz, 1H, NH-Et).

   Mass Spectrum (CI-NH$_3$): m/e 444 (MH$^+$, base).

15

Example 6

   This example describes the synthesis of N^6-
benzyladenosine-5'-N-methyluronamide.

   To a solution of adenosine-5'-N-methyluronamide

20   (MECA, 20 mg, 0.068 mmol) in DMF (1 ml) was added benzyl
bromide (24 µl, 0.20 mmol), and the solution was stirred
for 60 hrs at 40°C. DMF was removed under vacuum giving a
syrup that crystallized when acetone and ether were
added. The solvent was removed by decantation. Methanol

25   (0.5 ml) and concentrated K$_2$CO$_3$ (10 mg) were added, and
the mixture was warmed in a closed vessel at 60 °C for 6
hrs. The reaction mixture was cooled, filtered and
evaporated. The chromatographically pure product was
isolated using C18-silica packed cartridges. Yield:3.4

30   mg (13% yield).

   ^{1}H NMR (in DMSO-d$_6$): δ 2.7 (d, J=4 Hz, 3H, CH$_3$), 4.13
(t, J=4 Hz, 1H, H-3'), 4.31 (q, J=4.5 and 7, 1H, H-4'),
4.59 (m, 1H, H-2'), 4.71 (br. s, 2H, N^6-CH$_2$Ph), 5.96 (d,
J= 7 Hz, 1H, H-1'), 7.30 (m, 5H, Phenyl), 8.29 (s, 1H, H-

35   2), 8.43 (s, 1H, H-8), 8.56 (br. s, 1H, N^{6}H-CH$_2$Ph), 8.86
(d, J=4 Hz, 1H, NH-Me).

43

Example 7

This example describes the synthesis of N^6-benzyladenosine-5'-N-cyclopropyluronamide.

To a solution of adenosine-5'-N-cyclopropyluronamide (20 mg, 0.062 mmol) in anhydrous DMF (1 ml) was added benzyl bromide (22 μl, 0.19 mmol), and the solution was stirred for 60 hrs at 40°C. DMF was removed under vacuum giving a syrup that crystallized when acetone and ether were added. The solvent was removed by decantation. Methanol (0.5 ml) and concentrated NH_4OH (2.0 ml) were added, and the mixture was warmed in a closed vessel at 90 °C for 2 hrs. The mixture was reduced in volume by evaporation and cooled in an ice bath, resulting in precipitation of the chromatographically pure product. The white solid was isolated by filtration, washed with water, and dried to yield 15 mg (59% yield) of product which melted at 178-180°C.

^{1}H NMR (in DMSO-d_6): δ 0.46 (m 0, 2H, CH_2), 0.69 (m, 2H, CH_2), 2.70 (d, 1H, CH_3), 4.13 (t, J=4 Hz, 1H, H-3'), 4.27 (s, 1H, H-4'), 4.57 (c, 1H, H-2'), 4.71 (br. s, 2H, N^6-CH_2Ph), 5.53 (d, J= 8Hz, 1H, OH-2'), 5.73 (d, J= 4Hz, 1H, MeOH-3')5.95 (d, J=8 Hz, 1H, H-1'), 7.30 (m, 5H, Phenyl), 8.22 (s, 1H, H-2), 8.42 (s, 1H, H-8), 8.56 (br. s, 1H, N^6H-CH$_2$Ph), 8.88 (d, J=4 Hz, 1H, NH-Me).

Example 8

This example describes the synthesis of N^6-(2-nitrobenzyl)adenosine-5'-N-ethyluronamide.

To a solution of NECA (25 mg, 0.081 mmol) in DMF (0.5 ml) was added 2-nitrobenzyl bromide (53 mg, 0.249 mmol), and the solution was stirred for two days at 40°C. DMF was removed under vacuum giving a syrup that crystallized when acetone and ether were added. The solvent was removed using a Pasteur pipette. Methanol (2.0 ml) and concentrated NH_4OH (2.0 ml) were added, and the mixture was warmed in a closed vessel at 90 °C for 45 min. The solvent was evaporated, and the product was

44

purified by preparative TLC (silica gel, ethyl acetate: isopropanol:water, 4:1:2) to yield 3.5 mg of the pure product (9.7% yield). Mp: 181°C.

^{1}H NMR (in DMSO-d_4): δ 1.05 (t, J=7 Hz,3H, CH_3), 3.30 (m, 2H, CH_2), 4.13 (t, J=5 1Hz), 4.27 (m, 1H, H-3'), 4.30 (s, 1H, H-4"), 4.58 (m, 1H, H-2'), 4.98 (m, 2H, N^6-CH_2Ph),5.54 (d, J=7, 1H, OH-2'0, 5.73 (d, J=4, 1H, OH-4'), 5.97 (d, J=7, 1H, H-1'), 7.55 (m, 2H, arom), 7.67 (t, 1H, arom), 8.04 (d, 1H, arom), 8.21 (s, 1H, H-2'), 8.47 (s, 1H, H8), 8.62 (m, $1HN^6$H-CH_2Ph), 8.80 (t, J=5, 1H, NH-Et).

Example 9

This example describes the synthesis of N^6-(3-chlorobenzyl)adenosine-5'-N-ethyluronamide.

To a solution of NECA (50 mg, 0.162 mmol) in DMF (1 ml) was added 3-chlorobenzyl bromide (61 μl, 0.47 mmol), and the solution was stirred in a closed vessel for two days at 40°C. DMF was evaporated under a stream of nitrogen. The residue was treated with acetone (1 ml) and ether (2 ml). The solvent was removed using a Pasteur pipette, and the residue, an amorphous solid, was again extracted with chloroform to remove traces of the benzyl bromide. The dried residue was treated with methanol (2.0 ml) and concentrated NH_4OH (4.0 ml), and the mixture was warmed in a closed tube at 90 °C for 2 h with stirring. The mixture was reduced in volume by evaporation and cooled in an ice bath, resulting in precipitation of the chromatographically pure product. The white solid was isolated by filtration to yield 43 mg (62% yield) of product, which melted at 199-200°C.

^{1}H NMR (in DMSO-d_6): δ 1.06 (t, J=7 Hz,3H, CH_3), 3.2 (m, 2H, CH_2), 4.13 (t, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.6 (m, 1H, H-2'), 4.71 (br. s, 2H, N^6-CH_2Ph), 5.53 (d, J=7 Hz, 1H, OH-2'), 5.72 (d, J=4 Hz, 1H, OH-3'), 5.97 (d, J=8 Hz, 1H, H-1'), 7.2-7.4 (m, 4H, Phenyl), 8.26 (s, 1H, H-

45

2), 8.44 (s, 1H, H-8), 8.6 (br. s, 1H, N^{6}H-CH$_2$Ph), 8.82
(t, J=5 Hz, 1H, NH-Et).

Mass Spectrum (CI-NH$_3$): m/e 433 (MH$^+$, base).

Example 10

5   This example describes the synthesis of N^6-(4-
methoxybenzyl)adenosine-5'-N-ethyluronamide.

To a solution of NECA (50 mg, 0.162 mmol) in DMF
(0.5 ml) was added 4-methoxybenzyl chloride (33 μl, 0.24

10   mmol), and the solution was stirred for three days at
40°C. DMF was removed under vacuum giving a syrup that
crystallized when acetone and ether were added. The
solvent was removed using a Pasteur pipette. Methanol
(1.0 ml) and concentrated NH$_4$OH (2.0 ml) were added, and

15   the mixture was warmed in a closed vessel at 90 °C for 45
min. The solvent was evaporated, and the product was
purified by preparative TLC (silica gel, ethyl acetate:
isopropanol:water, 4:1:2) to yield 4.9 mg of the pure
product (14% yield).

20   ^{1}H NMR (in DMSO-d$_6$): δ 1.07 (t, J=7 Hz,3H, CH$_3$), 3.2
(m, 2H, CH$_2$), 3.69 (s, OCH$_3$), 4.12 (d, J=5 Hz, 1H, H-3'),
4.29 (s, 1H, H-4'), 4.59 (c, J=8Hz, J=5 Hz 1H, H-2'),
4.63 (br. s, 2H, N^6-CPh), 5.96 (d, J=8 Hz, 1H, H-1'),
6.84 (d, J= 9Hz, 2H, arom), 7.27 (d, J= 9Hz, 2H, arom),

25   8.25 (s, 1H, H-2), 8.40 (s, 1H, H-8), 8.45 (br. s, 1H,
N^{6}H-CH$_2$Ph), 8.85 (t, J=5 Hz, 1H, NH-Et).

Mass Spectrum (Cl-NH$_3$): m/e 429 (MH$^+$, base).

Example 11

30   This example describes the synthesis of inosine-5'-
N-methyluronamide.

2',3'-Isopropylideneinosine 5'-carboxylic acid (50
mg, 0.155 mmol), EDAC (59 mg, 0.31 mmol), and N-
hydroxysuccinimide (36 mg, 0.31 mmol) were dissolved in

35   DMF (1 ml). Methylamine was added, and the mixture was
stirred for 90 min. Water was then added, and the 6-
hydroxypurine-5'-N-methyl-carboxamidoriboside solid

46

residue was separated and dried in vacuo.   6-
hydroxypurine-5'-N-methyl-carboxamidoriboside was
purified by column chromatography (silica, eluted with
chloroform:methanol:25% ammonium hydroxide, 80:20:1) to
5   yield 49.8 mg (96%) of the pure product.   The
isopropylidene group was removed by adding hydrochloric
acid (1 N, 1 ml) and heating to 60 °C for 45 min or until
complete as judged by TLC.   Inosine-5'-N-methyluronamide
was purified using reverse phase cartridges.

10

Example 12

This example describes the synthesis of N^6-(3-
iodobenzyl)adenosine-5'-N-methyluronamide.

6-Chloropurine-5'-N-methyl-carboxamidoriboside (35
15   mg, 99 μmol), 3-iodobenzylamine hydrochloride (28 mg, 104
μmol), and triethylamine (41 μl, 0.30 mmol) were
dissolved in absolute ethanol (1 ml).   The solution was
stirred at 75 °C for 16 hours in a sealed vessel.   The
solvent was evaporated under a stream of nitrogen, and
20   water was added to remove the triethylammonium salt.   The
supernatant was removed and discarded, and the insoluble
residue containing intermediate 3-iodobenzyladenine-5'-N-
methylcarboxamidoriboside was used without further
purification.

25   Hydrochloric acid (1 N, 1 ml) was added, and the
resulting solution was heated to 60 °C for 4 hours.
After cooling in an ice bath, sodium bicarbonate solution
was added to neutralize.   A white solid formed and was
filtered, washed with water, and dried to yield 28 mg of
30   N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (55%
yield overall).

Example 13

This example describes the synthesis of
35   N^6-(3-aminobenzyl)adenosine-5'-N-methyluronamide.

47

3-Aminobenzylamine was prepared by catalytic
reduction of 3-nitrobenzylamine hydrochloride (5% Pd/C in
methanol).

2',3'-Isopropylidene-inosine 5'-carboxylic acid (1.1

5   g, 3.4 mmol, Olsson et al., J. Med. Chem., 29, 1683-1689
(1986)) was added to a solution of thionyl chloride (0.51
ml, 0.68 mmol) and dimethylformamide (0.26 ml) in
anhydrous chloroform (43 ml, dried over Al_2O_3). The
mixture was heated to reflux with the exclusion of

10 moisture for 6 h. After cooling the solvent was removed
in vacuo leaving a syrup, that was dissolved in
chloroform (12 ml). The solution was cooled to 0 °C and
2 ml of methylamine dissolved in 20 ml of chloroform was
added. After stirring for 15 min at < 10 °C, the

15 solution was extracted successively with HCl (0.1 N, 3 X
60 ml), sodium bicarbonate (0.5 M, 100 ml), and water (2
X 50 ml). The organic layer was dried ($MgSO_4$) and the
solvent evaporated leaving 632 mg of homogeneous (R_f =
0.75, chloroform:methanol:ammonium hydroxide, 80:20:1)

20 2',3'-isopropylidene-6-chloropurine-riboside-5'-methyl-
uronamide (53% yield).   NMR DMSO, d_6 δ 8.80 (s, 1H,
purine), 8.73 (s, 1H, purine), 7.48 (s, 1H, C4'), 6.50
(s, 1H, C1'), 5.4-5.5 (m, 2H, C2' and C3'), 2.16 (d, J =
4.7 Hz, 3H, NCH_3), 1.53 (s, 3H, i-Pr), 1.34 (s, 3H, i-

25 Pr).

2',3'-isopropylidine-6-chloropurine-riboside-5'-
methyl-uronamide (100 mg, 0.28 mmol) was dissolved in
ethanol (1.5 ml) and treated with 3-aminobenzylamine (47
mg, 0.30 mmol) and triethylamine (117 μl, 0.84 mmol).

30 The solution was heated at 80 °C for 12 h in an oil bath.
The solvent was evaporated, leaving a solid residue.
The residue was treated with 1 N HCl (1.0 ml), and the
mixture heated at 80 °C for 45 min. Sodium bicarbonate
solution was added until pH 7, and the mixture was

35 extracted 3X with ethyl acetate. The solvent was removed
under vacuum, and the residue was recrystallized from

48

methanol/water to provide 56 mg of the pure product (50%
yield).

Example 14

5   This example describes the synthesis of
N^6-(3-trifluoromethylbenzyl)adenosine-5'-N-
methyluronamide.

The compound was prepared as described above for
Example 12 using 3-trifluorobenzylamine, except that due
10   to aqueous insolubility, the hydrolysis on the
isopropylidene protecting group was carried out in a 1:1
mixture of 1 N HCl and methanol.

Example 15

15   This example describes the synthesis of
N^6-(4-sulfobenzyl)adenosine-5'-N-methyluronamide
triethylammonium salt.

p-Sulfobenzylamine was synthesized in 40% yield from
benzylamine and fuming sulfuric acid, by an adaptation of
20   the method described in Jacobson et al., J. Med. Chem.,
35, 4143-4149 (1992), and was recrystallized from a
solution in ammonium hydroxide, upon neutralization with
hydrochloric acid. NMR DMSO, d_6 δ 8.10 (br.s, 3H, NH_3^+),
7.62 (d, J = 8.1 Hz, 2H, o- to sulfo), 7.38 (d, J = 8.1
25   Hz, 2H, m- to sulfo), 4.02 (s, 2H, CH_2).

2',3'-Isopropylidene-6-chloropurine-riboside-5'-
methyluronamide (30 mg, 85 μmol, prepared as described in
Example 13), p-sulfobenzylamine (17 mg, 90 μmol), and
triethylamine (34 μl, 0.27 mmol) were combined in
30   absolute ethanol (1 ml) and heated to 90 °C for 3 days.
The mixture was filtered, and the filtrate was reduced in
volume by evaporation leaving a viscous syrup. The
isopropylidene-protected intermediate was purified (23.5
mg, recovered) on a TLC plate run in
35   chloroform:methanol:acetic acid (85:10:5). Hydrochloric
acid (1 ml, 1N) was added, and the solution was warmed at
60 °C for 40 min. After cooling and evaporation of the

solvent, methanol and ethyl acetate were added. The resulting precipitate was separated and dried to provide 14.3 mg (29% yield) of N^6-(4-sulfobenzyl)adenosine-5'-N-methyluronamide triethylammonium salt.

Example 16

This example describes the synthesis of N^6-(3-fluorobenzyl)adenosine-5'-N-ethyluronamide.

6-Chloropurine-5'-N-ethyl-carboxamidoriboside (30 mg, 81 μmol) and 3-fluorobenzylamine (10.8 mg, 86 μmol) were dissolved in absolute ethanol (1 ml). Triethylamine (17 μl, 0.12 mmol) was added and the solution was warmed at 80 °C for 16 h in a sealed vessel. No starting material (R_f 0.41, silica TLC plates, chloroform:methanol, 95:5) remained in the mixture. The solvent was evaporated under a stream of nitrogen, and water was added to remove the triethylammonium salt. The supernatant was removed and discarded, and the insoluble residue containing intermediate 3-fluorobenzyladenine-5'-N-ethylcarboxamidoriboside was used without further purification.

The isopropylidene group was removed by addition of hydrochloric acid as described in Example 11. The intermediate 3-fluorobenzyladenine-5'-N-ethylcarboxamidoriboside and product displayed R_f values of 0.51 and 0.10, respectively (silica, chloroform:methanol, 95:5). After cooling, sodium bicarbonate solution was added to neutralize. A white solid precipitated and was filtered, washed with water, and dried to yield 23 mg of N^6-(3-fluorobenzyl)adenosine-5'-N-ethyluronamide (68% yield overall).

Example 17

This example describes the synthesis of 2',3'-isopropylidene-adenosine- 5'-carboxylic acid.

2', 3'- Isopropylidene-adenosine (0.5 g, 1.6 mmol, Aldrich Chemical Co., St. Louis MO) was dissolved in

glacial acetic acid (11 ml) and chromium trioxide (0.222
g, 2.22 mmol) was added. A brown suspension was formed.
The color changed gradually to dark green. The suspension
was stirred at room temperature for 4 days. The dark
5   solid formed was filtered, washed with water and
crystallized from MeOH to afford a white solid (0.28 g,
54%). mp 257°C. ^{1}H NMR (DMSO-d$_6$) δ 8.24 (s, 1H, H-8), 8.08
(s, 1H, H-2), 7.28 (br.s, 2H, NH$_2$), 6.33 (s, 1H, H-1'),
5.50 (ABq, 2H, H-2', H-3'), 4.68 (s, 1H, H-4'), 1.52,1.35
10   (s, 3H, Me) ppm. High Res. MS calc'd. for C$_{13}$H$_{15}$N$_5$O$_5$:
321.1058; Found: 321.1073. IR (KBr) v :3000, 1706 cm^{-1}·

Example 18

This example describes the synthesis of 2',3'-
15   Isopropylidene-adenosine-5'-methyl-carboxylate.

An excess of diazomethane in ether (0.75 M) was
added dropwise over 0.5 hr to a suspension of 2',3'-
isopropylidene adenosine 5'- carboxylic acid (0.22 g,
0.68 mmol) in dioxane: MeOH (1:1, 50 ml), until a clear
20   yellowish solution was obtained. The reaction mixture
was stirred at room temperature for 1.5 h. Nitrogen was
bubbled through the solution until the yellow color
disappeared. The solvent was removed and the product was
dried under high vacuum to produce a white solid (0.211
25   g, 92%). mp 221-222°C. ^{1}H NMR (DMSO d$_6$) δ : 8.25 (s, 1H,
H-8), 8.05 (s, 1H, H-2), 7.32 (s, 2H, NH2), 6.38 (s, 1H,
H-1'), 5.61 (d, J= 6Hz, 1H, H-2'), 5.43 (d, J= 6Hz, 1H,
H-3'), 4.85 (s, 1H, H-4'), 3.29 (s, 3H, CO$_2$Me), 1.51,
1.34 (s, 3H, Me) ppm. High Res. MS calc'd. for
30   C14H17N5O5: 335.1238; Found: 335.1230.

Example 19

This example describes the synthesis of 1,3-di-*n*-
butyl-6-aminouracil.
35   N, N-Di-*n*-butylurea (34.45 g, 0.2 mmol), cyanoacetic
acid (19.56 g, 0.23 mol), and acetic anhydride (81.66 ml,

0.8 mol) were heated at 80 °C for 2 h under nitrogen and
solvent was removed by rotary evaporation to yield
N-(2-cyanoacetyl)-N,N'-dibutylurea. The
N-(2-cyanoacetyl)-N,N'-dibutylurea was dissolved in
5   methanol (100 ml) and 4N NaOH (100 ml) was added. The
reaction mixture was cooled for 30 min with stirring and
the solid was filtered and dried to yield 1,3-di-n-butyl-
6-aminouracil (47.04 g, 99.5%) as a colorless solid, m.p.
90.1-92.4 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.72-1.93 (m, 6 H, 2 x
10   CH$_3$, 1.18-1.33 (m, 4 H, 2 x CH$_2$), 1.39-1.52 (M, 4 H, 2 x
CH$_2$), 3.70 (t, 2 H, N-CH$_2$), 3.76 (t, 2 H, N-CH$_2$), 4.64 (s,
1 H, H-4), 6.77 (br s, exchangeable with D$_2$O, 2 H, NH$_2$).

Example 20

15   This example describes the synthesis of 6-amino-1,3-
di-n-butyl-5-nitroso-uracil.
To a mixture of 1,3-di-n-butyl-6-amino-uracil g,
112.8 mmol), glacial acetic acid (10 ml), and 6 N HCl
(18.8 ml) in water (500 ml) was added dropwise a solution
20   of sodium nitrite (7.78, g, 112.8 mmol) in water (30 ml)
on ice bath. The reaction mixture was stirred for 30
min. The violet solid was collected by filtration and
washed with water (2 x 20 ml), dried for 2 days at 60 °C
in a vacuum oven to yield 6-amino-1,3-di-n-butyl-5-
25   nitroso-uracil (24.99 g, 82.6%), m.p. 199-205 °C; ^{1}H NMR
(CDCl$_3$) δ 0.97-1.01 (m, 6 H, 2 x CH$_3$), 1.36-1.49 (m, 4 H,
2 x CH$_2$), 1.62-1.74 (m, 4 H, 2 x CH$_2$), 3.94 (t, J=7.8 Hz,
2 H, N-CH$_2$), 4.08 (t, J=7.5 Hz, 2 H, N-CH$_2$), 6.46 (br s, 2
H, NH$_2$). Anal. Calc'd for C$_{12}$H$_{20}$N$_4$O$_3$: C, 53.72; H, 7.51;
30   N, 20.88; Found: C, 54.37; H, 7.67; N, 20.65.

Example 21

This example describes the synthesis of 1,3-di-n-
butyl-xanthine.
35   A mixture of 6-amino-1,3-di-n-butyl-5-nitroso-uracil
(3.1 g, 11.6 mmol) and 5% Pd-C in dry DMF (30 ml) was
hydrogenated at 50 psi until it became a colorless

52

solution (about 2 h). The catalyst was filtered off
through Celite pad and the filtrate was mixed with 88%
formic acid (15 ml) and 1-(3-dimethylaminopropyl)-3-
ethyl-carbodiimide hydrochloride (1.1 g, 5.74 mmol) at 0
5   °C under nitrogen. The violet solution was stirred at
room temperature for 3 h. DMF and formic acid were
removed by rotary evaporation and the solid residue was
mixed with 2 N sodium hydroxide (50 ml, 100 mmol). The
reaction mixture was heated at reflux for 1 h. The
10   yellow solution was cooled and neutralized with 6N
hydrochloric acid to pH 3. The solid was collected by
filtration, washed with water, and dried to yield 1,3-di-
n-butyl-xanthine (2.3 g, 75%), m.p. 165.5-169 °C; ^{1}H NMR
(DMSO-d_6) δ 1.41-1.47 (m, 6 H, 2 x CH_3), 1.61-1.68 (m, 4
15   H, 2 x CH_2), 1.74-1.79 (m, 2 H, CH_2), 1.82-1.85 (m, 2 H,
CH_2), 3.84 (t, 2 H, N-CH_2), 3.98 (t, 2 H, N-CH_2), 8.10 (s,
1 H, H-8), 13.7 (br s, 1 H, NH).

Example 22
20   This example describes the synthesis of 1,3-di-n-
butyl-xanthine-7-β-D-ribofuranoside.
          A mixture of 1,3-di-n-butyl-xanthine (3 g, 11.35
mmol), ammonium sulfate (5 mg), and HMDS (20 ml) was
stirred in reflux for 1 h under nitrogen. HMDS was
25   removed by rotary evaporation in vacuo with exclusion of
moisture. The brown syrup was dissolved in dry
acetonitrile (70 ml). 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-
D-ribofuranoside (5.73 g, 11.36 mmol), potassium
nonaflate (15.35 g, 45.39 mmol), and trichlorosilane
30   (4.29 ml, 42.5 mmol) were added to the solution and the
reaction mixture was refluxed for 3 h under nitrogen.
Aqueous saturated sodium bicarbonate (30 ml) and
chloroform (30 ml) were added. After stirring for 30
min, two layers were separated and the water layer was
35   extracted with chloroform (300 ml). The combined organic
layer was washed with brine, dried over anhydrous $MgSO_4$,
filtered, and concentrated to dryness. Purification on

silica gel column chromatography (CHCl$_3$-MeOH, 250:1 →
100:1) yielded 1,3-dibutyl-7-(2,3,5-tribenzoyl-1-β-D-
ribofuranosyl)xanthine as pale yellow foam. ^{1}H NMR
(CDCl$_3$) δ 0.92-0.98 (m, 6 H, 2 x CH$_3$), 1.71-1.44 (m, 4 H,
2 x CH$_2$), 1.53-1.61 (m, 2 H, CH$_2$), 1.68-1.76 (m, 2 H, CH$_2$,
3.98 (t, J=7.6 Hz, 2 H, N-CH$_2$), 4.09 (t, J=7.5 Hz, 2 H,
N-CH$_2$), 4.71-4.82 (m, 2 H), 4.87 (dd, J=11.5 and 2.7 Hz,
1 H), 5.98-6.05 (m, 2 H, H-5'), 6.68 (d, J=4.9 Hz, 1 H,
H-1'), 7.34-7.62, 7.91-8.11 (m, 16 H, Ar and H-8).

A mixture of 1,3-dibutyl-7-(2,3,5-tribenzoyl-1-β-D-
ribofuranosyl)xanthine and NH$_3$/MeOH (80 ml) was stirred
at room temperature for 2.5 days. The volatiles were
removed and the residue was purified on silica gel column
chromatography (CHCl$_3$-MeOH, 20:1) to yield 1,3-di-n-
butylxanthine-7-β-ribofuranoside (3.5 g, 78.1%) as a
white solid, m.p. 139-140 °C; ^{1}H NMR (DMSO-d$_6$) δ 0.89
(pseudo t, J=7.4 and 7.3 Hz, 6 H, 2 x CH$_3$), 1.23-1.35 (m,
4 H, 2 x CH$_2$), 1.47-1.57 (m, 2 H, CH$_2$), 1.59-1.69 (m, 2 H,
CH$_2$), 3.54 (dd, J=12.1 and 3.7 Hz, 1 H), 3.68 (dd, J=12.1
and 3.7 Hz, 1 H), 3.84-3.94 (m, 2 H), 3.99 (t, J=7.2 Hz,
2 H), 4.08 (t, J=4.9 Hz, 1 H), 4.33 (t, J=4.8 Hz, 1 H),
5.05 (t, exchangeable with D$_2$O, 1 H, 5'-OH), 5.15 (d,
exchangeable with D$_2$O, 1 H, OH), 5.50 (d, exchangeable
with D$_2$O, 1 H, OH), 6.10 (d, J=4.8 Hz, 1 H, H-1'), 8.45
(s, 1 H, H-8). Anal. Calc'd for C$_{18}$H$_{28}$N$_4$O$_6$: C, 54.53; H,
7.12; N, 14.13; Found: C, 54.71; H, 7.10; N, 14.10.

Example 23

This example describes the synthesis of 1,3-di-n-
butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside.

A mixture of 1,3-di-n-butylxanthine-7-β-D-
ribofuranoside (1.56 g, 3.94 mmol), p-toluenesulfonic
acid (1.3 g, 1.58 mmol), and dry acetone (25 ml) was
stirred at room temperature for 7 h and kept in
refrigerator for 2 days. After stirring for 1 h at room
temperature, the reaction mixture was neutralized by
triethylamine and the solvent was removed by rotary

54

evaporation. The residue was purified by silica gel
column chromatography (CHCl$_3$-MeOH, 100:0 → 50:1) to yield
1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuranoside (1.7 g, 98.9%) as a thick syrup. ^{1}H NMR
5   (DMSO-d$_6$) δ 0.89 (t, *J*=7.3 Hz, 6 H, 2 x CH$_3$), 1.21-1.35
(m, 7 H, 2 x CH$_2$ and isopropylidene), 1.47-1.56 (m, 5 H,
CH$_2$ and isopropylidene), 1.59-1.69 (m, 2 H, CH$_2$), 3.49-
3.57 (m, 2 H, H-5'), 3.86 (pseudo t, *J*=7.6 and 7.2 Hz, 2
H, N-CH$_2$), 3.99 (pseudo t, *J*=7.3 and 7.2 Hz, 2 H, N-CH$_2$),
10   4.17 (dd, *J*=8.1 and 4.9 Hz, 1 H), 4.88 (dd, *J*=6.4 and 3.1
Hz, 1 H), 5.07 (t, *J*=5.2 Hz, exchangeable with D$_2$O, 1 H,
5'-OH), 5.15 (dd, *J*=6.4 and 3.5 Hz, 1 H), 6.29 (d, *J*=3.0
Hz, 1 H, H-1'), 8.40 (s, 1 H, H-8). Anal. Calc'd for
C$_{21}$H$_{32}$N$_4$O$_6$: C, 57.78; H, 7.39; N, 12.84; Found: C, 57.68;
15   H, 7.43; N, 12.77.

Example 24

     This example describes the synthesis of methyl-1,3-
di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-
20   ribofuronate.

     A solution of sodium periodate (1.61 g, 7.53 mmol)
in water (15 ml) was added to a solution of 1,3-di-*n*-
butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside
(0.8 g, 1.83 mmol) in CHCl$_3$:CH$_3$CN(1:1,20 ml) at room
25   temperature and followed by ruthenium (III) chloride (3.8
mg, 0.018 mmol). The reaction mixture was stirred
vigorously for 6 days at room temperature. After the two
layers were separated, the aqueous layer was extracted
with chloroform (2 x 50 ml) and the combined organic
30   layer was washed with brine, dried over anhydrous MgSO$_4$,
filtered, and concentrated to dryness to yield crude 1,3-
di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronic
acid (0.74 g) as a foam.
     To a solution of acid (dried *in vacuo* for 3 h) in
35   MeOH (15 ml) were added DMAP (0.02 g, 016 mmol) and then
EDAC (0.79 g, 4.13 mmol) and the reaction mixture was
stirred for 17 h at room temperature. After removal of

solvent, the residue was dissolved in ethyl acetate (70
ml) and washed with water (2 x 30 ml), and brine (40 ml),
dried over anhydrous $MgSO_4$, filtered, and concentrated to
dryness. The residue was purified by silica gel column

5   chromatography (hexanes-ethyl acetate, 1:1) to yield
methyl-1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuronate (0.36 g, 47.2%) as a colorless solid and
1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuranoside (0.24 g) was recovered, m.p. 72-73 °C; [1]H

10   NMR ($CDCl_3$) δ 0.92-0.99 (m, 6 H, 2 x CH_3), 1.32-1.47 (m, 7
H, 2 x CH_2 and isopropylidene), 1.55-1.69 (m, 5 H, CH_2 and
isopropylidene), 1.71-1.79 (m, 2 H, CH_2), 3.77 (s, 3 H, -
OCH_3), 3.95 (t, *J*=7.8 Hz, 2 H, N-CH_2), 4.10 (pseudo t, *J*=
7.6 and 7.3 Hz, 2 H, N-CH_2), 4.80 (s, 1 H), 5.17 (d,

15   *J*=6.2 Hz, 1 H), 5.46 (d, *J*=6.2 Hz, 1 H), 6.26 (s, 1 H, H-
1'), 7.88 (x, 1 H, H-8). Anal. Calc'd for $C_{22}H_{32}N_4O_7$: C,
56.89; H, 6.94; N, 12.06; Found: C, 57.17; H, 7.07; N,
11.89.

20   Example 25
        This example describes the synthesis of methyl-1,3-
di-*n*-butylxanthine-7-β-D-ribofuronate.
        A solution of methyl-1,3-di-*n*-butylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (30 mg. 0.065 mmol) in

25   88% formic acid (3 ml) was stirred for 3 h at room
temperature and the solvent was removed by rotary
evaporation. The residue was purified by preparative TLC
($CHCl_3$-MeOH, 10:1) to yield methyl 1,3-di-*n*-
butylxanthine-7-β-D-ribofuronate (14.8 mg, 57%) as a

30   colorless solid, m.p. 178-179 °C; ^{1}H NMR (DMSO-d_6) δ 0.90
and 0.91 (2 x t, *J*=7.5 Hz, 2 x 3 H, 2 x CH_3), 1.23-1.35
(m, 4 H, 2x CH_2), 1.47-1.57 (m, 2 H, CH_2), 1.60-1.70 (m, 2
H, CH_2), 3.72 (s, 3 H,-OCH_3), 3.87 (t, *J*=7.3 Hz, 2 H, N-
CH_2), 4.00 (t, *J*=7.2 Hz, 2 H, N-CH_2), 4.29 (m, 1 H), 4.49

35   (m, 2 H), 5.73 (d, *J*=6.1 Hz, exchangeable with D_2O, 1 H,
OH), 5.81 (d, *J*=4.7 Hz, exchangeable with D_2O, 1 H, OH),
6.29 (d, *J*=5.4 Hz, 1 H, H-1'), 8.46 (s, 1 H, H-8). Anal.

56

Calc'd for $C_{19}H_{28}N_4O_7$: C, 53.77; H, 6.65; N, 13.20; Found:
C, 53.90; H, 6.62; N, 12.89.

Example 26

5   This example describes the synthesis of N-methyl
1,3-di-n-butylxanthine-7-β-D-ribofuronamide.

        To a solution of methyl-1,3-di-n-butyl-xanthine-7-β-
D-2,3-O-isopropylidene-ribofuronate (50 mg, 0.11 mmol) in
MeOH (10 ml) was bubbled methylamine for 5 min at -78 °C
10   (1/3 increase in volume). The reaction mixture was
heated for 17 h at 85 °C in a sealed tube. After
evaporation of the solvent, the slightly yellow residue
was purified by preparative TLC ($CHCl_3$-MeOH, 20:1) to
yield N-methyl 1,3-di-n-butylxanthine-7-β-D-2,3-
15   isopropylidene-ribofuronamide (23.1 mg, 46.3%) as a foam.
^1H NMR ($CDCl_3$) δ 0.96 and 0.97 (2 x t, J=7.3 Hz, 2 x 3 H,
2 x CH_3, 1.34-1.44 (m. 7 H, 2 x CH_2 and isopropylidene),
1.51-1.69 (m, 5H, CH_2 and isopropylidene), 1.72-1.79 (m,
2 H, CH_2), 2.79 (d, J=4.9 Hz, 3 H, -NH-$\underline{CH}_3$), 4.01 (t,
20   J=7.5 Hz, 2 H, N-CH_2), 4.12 (pseudo t, J=7.6 and 7.3 Hz,
2 H, N-CH_2), 4.60 (d, J=3.0 Hz, 1 H), 5.14 (dd, J=6.9 and
4.0 Hz, 1 H), 5.21 (dd, J=6.9 and 3.1 Hz, 1 H), 5.91 (d,
J=4.0 Hz, 1 H, H-1'), 6.92 (m, 1 H, NH), 7.72 (s, 1 H, H-
8).

25   A mixture of isopropylidene compound (20 mg, 0.043
mmol) and 88% formic acid (3 ml) was reacted for 6 h at
room temperature. After the reaction mixture was
concentrated to dryness, the residue was coevaporated
with toluene (2 x 5 ml) and triturated with ether to
30   yield N-methyl 1,3-di-n-butylxanthine-7-β-D-
ribofuronamide (12.7 mg, 69.5%) as a colorless solid,
m.p. 180-181 °C; ^1H NMR (DMSO-d_6) δ 0.88-0.92 (m, 6 H, 2 x
CH_3), 1.26-1.35 (m, 4 H, 2 x CH_2), 1.48-1.58 (m, 2 H,
CH_2), 1.61-1.70 (m, 2 H, CH_2), 2.64 (d, J=4.3 Hz, 3 H,
35   NHCH_3), 3.88 (t, J=7.4 Hz, 2 H, N-CH_2), 4.01 (pseudo t,
J=7.3 and 7.1 Hz, 2 H, N-CH_2), 4.16-4.19 (m, 1 H), 4.30
(d, J=3.6 Hz, 1 H), 4.44-4.47 (m, 1 H), 5.60 (pseudo t,

57

J=6.5 and 5.5 Hz, exchangeable with D_2O, 2 H, 2 x OH),
6.19 (d, J=5.4 Hz, 1 H, H-1'), 8.11 (q, J=4.3 Hz,
exchangeable with D_2O, 1 H, NH), 8.65 (s, 1 H, H-8).

5   Example 27

This example describes the synthesis of 1,3-di-*n*-
butylxanthine-7-β-D-ribofuronamide.

A mixture of methyl-1,3-di-*n*-butylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (87 mg, 0.19 mmol) and
10   methanolic ammonia (10 ml, saturated at 0 °C) was stirred
at 85 °C for 18 h in a sealed bottle. After cooling, the
volatiles were removed by rotary evaporation and the
residue was purified by preparative TLC ($CHCl_3$-MeOH,
20:1) to yield 1,3-di-*n*-butyl-xanthine-7-β-D-2,3-
15   isopropylidene-ribofuronamide (67.5 mg, 80.2%) as a
syrup. ^{1}H NMR ($CDCl_3$) δ 0.93-0.99 (m, 6 H, 2 x CH_3), 1.32-
1.47 (m, 7 H, 2 x CH_2 and isopropylidene), 1.55-1.66 (m,
5 H, CH_2 and isopropylidene), 1.69-1.79 (m, 2 H, CH_2),
4.00 (pseudo t, J=7.8 and 7.3 Hz, 2 H, N-CH_2), 4.12
20   (pseudo t, J=7.5 and 7.3 Hz, 2 H, N-CH_2), 4.59 (d, J=3.6
Hz, 1 H), 5.15 (dd, J=7.1 and 3.9 Hz, 1 H), 5.28 (dd,
J=7.1 and 3.6 Hz, 1 H), 5.35 and 6.86 (2 x br s, 2 x 1 H,
NH_2'), 5.93 (d, J=3.9 Hz, 1 H, H-1'), 7.74 (s, 1 H, H-8).

A similar deisopropylidenation procedure for N-
25   methyl- 1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide with
56 mg of protected compound followed by crystallization
with ether-MeOH yielded 1,3-di-*n*-butylxanthine-7-β-D-
ribofuronamide (20 mg, 40%) as a slightly yellow solid,
m.p. 158, 4 °C; ^{1}H NMR (DMSO-d_6) δ 0.88-0.92 (m, 6 H, 2 x
30   CH_3), 1.23-1.37 (m, 4 H, 2 x CH_2), 1.47-1.57 (m, 2 H,
CH_2), 1.60-1.70 (m, 2 H, CH_2), 3.87 (pseudo t, J=7.5 and
7.2 Hz, 2 H, N-CH_2), 4.00 (pseudo t, J=7.3 and 7.2 Hz, 2
H, N-CH_2), 4,14-4.15 (m, 1 H, H-3'), 4.29 (d, J=3.7 Hz, 1
H, H-4'), 4.40-4.43 (m, 1 H, H-2'), 5.58 (d, J=5.0 Hz,
35   exchangeable with D_2O, 1 H, OH), 5.62 (d, J=6.1 Hz,
exchangeable with D_2O, 1 H, OH), 6.19 (d, J=5.8 Hz, 1 H,

58

H-1'), 7.43 and 7.62 (2 x br s, exchangeable with D_2O, 2
x 1 H, NH_2), 8.67 (s, 1 H, H-8).

Example 28

5   This example describes the synthesis of N-ethyl-1,3-
di-*n*-butyl-xanthine-7-β-D-ribofuronamide.

A mixture of methyl-1,3-di-*n*-butylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (70 mg, 0.15 mmol) and
25% ethylamine/MeOH (10 ml, dissolved at -78 °C) was
10   heated for 18 h at 85 °C in a sealed tube. After
evaporation of the solvent, the slightly yellow residue
was purified by preparative TLC ($CHCl_3$-MeOH, 20:1) to
yield N-ethyl-1,3-di-*n*-butyl-xanthine-7-β-D-2,3-
isopropylidene-ribofuronamide (55.7 mg, 77.4%) as a
15   syrup. ^1H NMR ($CDCl_3$) δ 0.93-0.99 (m, 6 H, 2 x CH_3), 1.11
(t, J=7.3 Hz, 3 H,-$NHCH_2CH_3$), 1.33-1.41 (m, 7 H, 2 x CH_2
and isopropylidene), 1.58-1.70 (m, 5 H, CH_2 and
isopropylidene), 1.72-1.77 (m, 2 H, CH_2), 3.24-3.32 (m, 2
H,-$NHCH_2CH_3$), 4.00 (pseudo t, J=7.9 and 7.8 Hz, 2 H, N-
20   CH_2), 4.12 (pseudo t, J=7.5 and 7.3 Hz, 2 H, N-CH_2), 4.58
(d, J=3.5 Hz, 1 H), 5.13 (dd, J=6.7 and 3.8 Hz, 1 H),
5.18 (dd, J=6.7 and 3.1 Hz, 1 H), 5.91 (d, J=4.2 Hz, 1 H,
H-1'), 6.96 (m, 1 H, NH), 7.73 (s, 1 H, H-8).

A similar deisopropylidenation procedure for N-
25   methyl 1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide with
55 mg of protected compounds followed by crystallization
with ether-MeOH yielded N,N-dimethyl-1,3-di-*n*-
butylxanthine-7-β-D-ribofuronamide (25.1 mg, 73.8%) as a
colorless solid, m.p. 189.4-189.8 °C; ^1H NMR (DMSO-d_6) δ
30   0.88-0.93 (m, 6 H, 2 x CH_3), 1.05 (t, J=7.4 Hz, 3 H,
$NHCH_2CH_3$), 1.24-1.35 (m, 4 H, 2 x CH_2), 1.48-1.58 (m, 2 H,
CH_2), 1.61-1.71 (m, 2 H, CH_2), 3.09-3.19 (m, 2 H,
$NHCH_2CH_3$), 3.88 (pseudo t, J=7.5 and 7.2 Hz, 2 H, N-CH_2),
4.01 (t, J=7.2 Hz, 2 H, N-CH_2), 4.14-4.19 (m, 1 H), 4.29
35   (d, J=3.5 Hz, 1 H), 4.43-4.48 (m, 1 H), 5.57 and 5.59 (2
x d, J=6.4 and 5.5 Hz, exchangeable with D_2O, 2 H, 2 x

59

OH), 6.17 (d, J=5.5 Hz, 1 H, H-1'), 8.18 (t, J=5.4 Hz, exchangeable with D_2O, 1 H, NH), 8.64 (s, 1 H, H-8).

Example 29

This example describes the synthesis of N,N-dimethyl-1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide.

A solution of methyl-1,3-di-*n*-butyl-xanthine-7-β-D-2,3-O-isopropylidene-ribofuronate. (77 mg, 0.166 mmol) and 25% dimethylamine/MeOH (10 ml, dissolved at -78 °C) was heated for 15 h at 75 °C in a sealed tube. After evaporation of the volatiles, the residue was purified by preparative TLC ($CHCl_3$-MeOH, 20:1) to yield, N,N-dimethyl-1,3-di-*n*-butylxanthine-7-β-D-2,3-isopropylidene-ribofuronamide (50 mg, 63.2%) as a thick syrup. ^{1}H NMR ($CDCl_3$) δ 0.92-0.98 (m, 6 H, 2 x CH_3), 1.33-1.46 (m, 7 H, 2 x CH_2 and isopropylidene), 1.58-1.68 (m, 5 H, CH_2 and isopropylidene), 1.71-1.78 (m, 2 H, CH_2), 2.93 and 3.11 [2 x s, 2 x 3 H, -N(CH_3)$_2$], 4.01 (t, J=7.8 Hz, 2 H, N-CH_2), 4.09 (t, J=7.6 Hz, 2 H, N-CH_2), 5.07 (s, 1 H), 5.15 (d, J=6.3 Hz, 1 H), 5.33 (m, 1 H), 6.69 (s, 1 H, H-1'), 8.01 (s, 1 H, H-8).

A similar deisopropylidenation procedure for N-methyl 1,3-di-*n*-butyl-xanthine-7-β-D-ribofuronamide with 40 mg of protected compound followed by purification on preparative TLC ($CHCl_3$-MeOH, 10:1) yielded N,N-dimethyl-1,3-di-*n*-butyl-xanthine-7-β-D-ribofuronamide (30 mg, 82%) as a colorless solid, m.p. 154.4-154.9 °C; ^{1}H NMR (DMSO-d_6) δ 0.88-0.92 (m, 6 H, 2 x CH_3), 1.24-1.37 (m, 4 H, 2 x CH_2), 1.48-1.57 (m, 2 H, CH_2), 1.60-1.70 (m, 2 H, CH_2), 2.89 and 3.05 [2 x s, 2 x 3 H, N(CH_3)$_2$], 3.87 (pseudo t, J=7.5 and 7.1 Hz, 2 H, N-CH_2, 4.00 (pseudo t, J=7.2 and 6.9 Hz, 2 H, N-CH_2), 4.22 (dd, J=8.7 and 4.2 Hz, 1 H), 4.29-4.34 (m, 1 H), 4.90 (d, J=3.9 Hz, 1 H), 5.65 and 5.69 (2 x d, J=5.5 and 6.0 Hz, exchangeable with D_2O, 2 H, 2 x OH), 6.32 (d, J=4.7 Hz, 1 H, H-1'), 8.75 (s, 1 H, H-8).

60

Example 30

This example describes the synthesis of 1-O-methyl 5-(*t*-butyldiphenylsilyl)-β-D-ribofuranoside.

To a mixture of methyl-β-D-ribofuranoside (460 mg,
5   2.8 mmol, Sigma, St. Louis, MO) and anhydrous methylene chloride (20 ml) were added triethylamine (0.468 ml, 3.36 mmol), *t*-butyldiphenylchlorosilane (0.9 ml, 3.46 mmol), and DMAP (13.7 mg, 0.112 mmol) successively at room temperature. The reaction mixture was stirred at room
10 temperature for 18 h under nitrogen. It was washed with water (20 ml), saturated ammonium chloride (20 ml), and brine (20 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was separated by silica gel column chromatography ($CHCl_3$-MeOH, 50:1) to
15 yield 1-O-methyl 5-(*t*-butyldiphenylsilyl)-β-D-ribofuranoside (618 mg, 54.8%) as a thick syrup. ^{1}H NMR (DMSO-d_6) δ 0.96 (s, 9 H, *t*-Bu), 3.22 (s, 3 H,-OCH_3), 3.61 (dd, *J*=11.0 and 5.3 Hz, 1 H), 3.74 (d, *J*=4.4 Hz, 1 H), 3.81 (dd, *J*=11.0 and 2.7 Hz, 1 H), 3.90 (m, 1 H), 4.00
20 (m, 1 H), 4.68 (s, 1 H, H-1'), 4.84 (br s, 1 H, exchangeable with D_2O, OH), 5.05 (br s, 1 H, exchangeable with D_2O, OH), 7.45 and 7.67 (m, 10 H, Ph_2).

Example 31

25   This example describes the synthesis of 1-O-methyl 5-(*t*-butyldiphenylsilyl)-2,3-dibenzoyl-β-D-ribofuranoside.

To a solution of 1-O-methyl-5-(*t*-butyldiphenylsilyl)-β-D-ribofuranoside (579 mg, 1.44
30 mmol) in methylene chloride-pyridine (4:1, 12.5 ml) was added dropwise benzoyl chloride (0.367 ml, 3.16 mmol) at 0 °C. The reaction mixture was stirred for 2.5 h at 0 °C and for 14.5 h at room temperature. Ice was added to quench the reaction and it was stirred for 1 h.
35 Methylene chloride (100 ml) was added and two phases were separated. The organic layer was washed with water, saturated ammonium chloride, and brine, dried over $MgSO_4$,

filtered, and concentrated to dryness to yield crude 1.0-methyl 5-(*t*-butyldiphenylsilyl)-2,3-dibenzoyl-β-D-ribofuranoside), which was purified by silica gel column chromatography (hexanes-ethyl acetate, 5:1 → 1:1) to yield 1.0-methyl 5-(*t*-butyldiphenylsilyl)-2,3-dibenzoyl-β-D-ribofuranoside (869 mg, 99%) as thick syrup. ^{1}H NMR (CDCl$_3$) δ 1.05 (s, 9 H, *t*-Bu), 3.42 (s, 3 H,-OCH$_3$), 3.86 (dd, *J*=11.1 and 4.8 Hz, 1 H, H-5a), 3.92 (dd, *J*=11.1 and 4.7 Hz, 1 H, H-5b), 4.48 (dd, *J*=10.6 and 4.6 Hz, 1 H), 5.15 (s, 1 H), 5.63 (d, *J*=5.0 Hz, 1 H), 5.82 (pseudo t, *J*=5.8 and 5.4 Hz, 1 H), 7.29-8.18 (m, 20 H, Ar).

Example 32

This example describes the synthesis of 1-O-methyl 2,3-dibenzoyl-β-D-ribofuranoside.

A solution of 1-O-methyl-5-(*t*-butyldiphenylsilyl)-2,3-dibenzoyl-β-D-ribofuranoside (849 mg, 1.39 mmol) and 1.0 M tetrabutylammonium fluoride in THF (1.53 ml, 1.53 mmol) was stirred for 2 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography (hexanes-ethyl acetate, 1:1) to yield 1-O-methyl-2,3-dibenzoyl-β-D-ribofuranoside (461 mg, 89%) as thick syrup. ^{1}H NMR (DMSO-d$_6$) δ 3.38 (s, 3 H,-OCH$_3$), 3.62 (m, 2 H, H-5), 4.37 (q, *J*=5.2 Hz, 1 H, H-4), 5.02 (pseudo t, *J*=6.0 and 5.3 Hz, exchangeable with D$_2$O, 1 H, 5-OH), 5.18 (s, 1 H, H-1), 5.45 (m, 1 H, H-2), 5.53 (t, *J*=5.2 Hz, 1 H, H-3), 7.42-7.88 (m, 10 H, Ar).

Example 33

This example describes the synthesis of N-methyl-1-O-methyl-2,3-dibenzoyl-β-D-ribofuronamide.

A mixture of 1-O-methyl-2,3-dibenzoyl-β-D-ribofuranoside (374.8 mg, 1.01 mmol), ruthenium (IV) oxide (10 mg), and solium periodate (1161 mg, 5.43 mmol) in CHCl$_3$:CH$_3$CN:H$_2$O (2:2:3, 14 ml) was stirred vigorously for 2.5 h at room temperature. Chloroform (20 ml) was added and semisolid was removed by filtration. The

filtrate was separated and the water layer was extracted
with chloroform (2 x 40 ml). Combined washings and
extracts were washed with brine, dried over $MgSO_4$,
filtered, and concentrated to dryness. After drying *in
vacuo* overnight 1-O-methyl-2,3-dibenzoyl-β-D-ribofuronic
acid (340 mg, 89.5%) was obtained as a thick syrup. [1]H
NMR ($CDCl_3$) δ 3.56 (s, 3 H,-OCH_3), 4.90 (d, *J*=6.1 Hz, 1 H,
H-4), 5.25 (s, 1 H, H-1), 5.66 (d, *J*=5.0 Hz, 1 H, H-2),
6.00 (pseudo t, *J*=5.7 and 5.4 Hz, 1 H, H-3), 7.32-7.99
(m, 10 H, Ar).

EDAC (198 mg, 1.04 mmol) was added to a solution of
acid (0.16 g, 0.414 mmol) in MeOH (3 ml) and the reaction
mixture was stirred for 3 h at room temperature. After
the solvent was removed by rotary evaporation, the
residue was dissolved in chloroform (50 ml) and it was
washed with water (30 ml) and brine (30 ml), dried over
anhydrous $MgSO_4$, filtered, and concentrated to dryness.
The residue was purified by preparative TLC (hexanes-
ethyl acetate, 1:1) to yield methyl 1-O-methyl-2,3-
dibenzoyl-β-D-ribofuronate (120 mg, 72.3%) as colorless
solid, m.p. 92.2-93.7 °C; ^{1}H NMR ($CDCl_3$) δ 3.52 (s, 3 H,
1-OCH_3), 3.82 (2, 3 H, 5-OCH_3), 4.84 (d, *J*=6.2 Hz, 1 H, H-
4), 5.21 (s, 1 H, H-1), 5.62 (d, *J*=4.8 Hz, 1 H, H-2),
6.01 (pseudo t, *J*=5.7 and 5.5 Hz, 1 H, H-3), 7.32-7.99
(m, 10 H, Ar).

A mixture of methyl ester (35 mg, 0.087 mmol) in 2.0
M methylamine in THF (3 ml) was heated for 15 h at 50 °C
in a sealed tube. The volatiles were removed by
evaporation and the residue was reacted with benzoyl
chloride (0.15 ml, 1.29 mmol) in methylene chloride-
pyridine (2:1, 6 ml) for 3 h at room temperature. Ice
was added and the reaction mixture was stirred for 15
min. Methylene chloride (30 ml) was added and the two
layers were separated. The aqueous layer was extracted
with methylene chloride (20 ml) and the combined organic
layer was washed with brine (30 ml), dried over $MgSO_4$,
filtered, and concentrated to dryness. The residue was

63

separated by preparative TLC (hexanes-ethyl acetate, 1:1) to yield 1,3-di-n-butyl-xanthine (25 mg, 72%) as a syrup. ^1H NMR ($CDCl_3$) δ 2.92 (d, J=5.0 Hz, 3 H, -NHCH$_3$), 3.58 (s, 3 H, 1-OCH$_3$), 4.82 (d, J=5.5 Hz, 1 H, H-4), 5.24 (s, 1 H, H-1), 5.60 (m, 1 H, H-2), 5.87 (t, J=5.1 Hz, 1 H, H-3), 6.68 (br m, 1 H, -NH), 7.33-7.99 (m, 10 H, Ar).

Example 34

This example describes the culture of Chinese hamster ovary (CHO) cells and the preparation of a suspension of CHO cell membranes stably transfected with rat A$_3$ cDNA. These materials were used for the subsequent experimental work set out herein.

CHO cells were transfected with rat A$_3$ cDNA (Meyerhof et al., supra) using methods well known to those of skill in the art. CHO cells stably expressing the A$_3$ receptor (Zhou et al., supra) were grown in F-12 (Ham's) medium (Gibco BRL, Gaithersburg, MD) containing 10% fetal bovine serum (FBS, Gibco BRL) and penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively; Gibco BRL) at 37 °C in a 5% CO_2 atmosphere. When the transfected CHO cells had reached confluency, they were washed twice with Dulbecco's phosphate buffer solution before dislodging after addition of 3 ml trypsin-EDTA. For the final passage, cells were grown in 150 x 50 mm tissue culture dishes. Cells were washed twice with 10 ml of ice-cold lysis buffer (10 mM Tris, 5 mM EDTA, pH 7.4, 5 °C). After addition of 5 ml of lysis buffer, cells were mechanically scraped and homogenized in an ice-cold Dounce homogenizer (20 strokes by hand). The suspension was centrifuged at 43,000 x g for 10 min. The pellet was resuspended in the minimum volume of ice-cold 50/10/1 buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 8.26, 5 °C) required for the binding assay and homogenized in a Dounce homogenizer. Typically, 6-8 175 cm^2 flasks were used for a 48-tube assay. Adenosine deaminase (ADA, Boehringer Mannheim, Indianapolis, IN)

64

was added to a final concentration of 3 U/ml, and the
suspension was incubated at 37 °C for 15 min. The
membrane suspension was subsequently kept on ice until
use. When large batches (ca 100 flasks) were processed,

5   homogenization was performed with a Polytron (Brinkman,
Luzern, Switzerland) and further work-up was as described
above. The preparation was stored at -70 °C and retained
its [^{125}I]N^6-2-(4-aminophenyl)ethyladenosine ([^{125}I]APNEA,
prepared as described in Stiles et al., J. Biol. Chem.,

10   260, 10806-10811 (1985)) binding properties for at least
one month.

     Rat cerebral cortical and striatal membranes were
prepared (Jacobson et al., J. Med. Chem., 35, 4143-4149
(1992)) and treated with ADA for 30 min at 37 °C prior to

15   storage at -70°C.

                        Example 35
     This example describes a radioligand binding assay
used to study the structure activity relationship (SAR)

20   at the A$_3$ receptor.
     Binding of [^{125}I]APNEA to CHO cells stably
transfected with the A$_3$ receptor clone was performed
essentially as described in Stiles et al., J. Biol.
Chem., 260, 10806-10811 (1985). Assays were performed in

25   50/10/1 buffer in glass tubes which contained 100 μl of
the membrane suspension, 50 μl of [^{125}I]APNEA (final
concentration 0.5 nM) or [^{125}I]AB-MECA and 50 μl of
inhibitor. Inhibitors were routinely dissolved in
dimethylsulfoxide (DMSO) and were then diluted with

30   buffer. The final DMSO concentrations never exceeded 1%;
this concentration did not influence [^{125}I]APNEA binding.
Incubations were carried out in duplicate for 1 hour at
37 °C, and were terminated by rapid filtration over
Whatman GF/B filters, using a Brandell cell harvester

35   (Brandell, Gaithersburg, MD). Tubes were washed three
times with 3 ml of buffer. Radioactivity was determined
in a Beckman gamma 5500B γ-counter. Non-specific binding

65

was determined in the presence of 40 μM N^6-[(R)-1-methyl-
2-phenylethyl]adenosine (R-PIA). K_i-values were
calculated according to Cheng-Prusoff (Cheng et al.,
<u>Biochem. Pharmacol.</u>, <u>22</u>, 3099-3108 (1973)), assuming a K_d

5   for [^{125}I]APNEA of 17 nM (Zhou et al., <u>supra</u>).

Binding of [^{3}H]PIA (Amersham, Arlington Heights, IL)
to A_1 receptors from rat brain membranes and of [^{3}H]CGS
21680 (DuPont NEN, Boston, MA) to A_2 receptors from rat
striatal membranes was performed as described previously

10   (Jacobson et al. (1992), <u>supra</u>).

Solid samples of the adenosine derivatives were
dissolved in DMSO and stored in the dark at -20°C. The
stock solutions were diluted with DMSO to a concentration
of $\leq$ 0.1 mM prior to addition to the aqueous medium. The

15   final concentration of DMSO in the assay medium was
generally 2%.

Binding data for a variety of adenosine derivatives,
as well as a number of nucleosides having bases other
than adenine, are set forth in the table below. At least

20   six different concentrations spanning three orders of
magnitude, adjusted appropriately for the IC_{50} of each
compound, were used. IC_{50} values, computer-generated
using a nonlinear regression formula of the GraphPAD
program (Institute of Scientific Information), were

25   converted to apparent K_i values using K_D values (Jacobson
et al. (1992), <u>supra</u>) of 1.0 and 14 nM for [^{3}H]PIA and
[^{3}H]CGS 21680 binding, respectively, and the Cheng-
Prusoff equation (Cheng et al., <u>Biochem. Pharmacol.</u>, <u>22</u>,
3099-3108 (1973)).

Table 1: Affinities of selected compounds at A_1, A_2 and A_3 receptors indicated as either K_i (nM) or percent displacement at a concentration of 100 μM, unless otherwise indicated. Values are means of 3-5 experiments ± SEM. Values without SEM are taken from the literature.

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
		purine and 5'-modified adenosines		
1	ADAC	0.85 nM	210 nM	281 ± 51 nM
2	R-PIA	1.2 nM	124 nM	158 ± 52 nM
3	S-PIA	49.3 nM	1,820 nM	920 ± 311 nM
4	CPA	0.59 nM	462 nM	240 ± 36 nM
5	CHA	1.3 nM	514 nM	167 ± 26 nM
6	N^6-phenyladenosine	4.62 nM	663 nM	802 ± 279 nM
7	N^6-benzyladenosine	120 nM	285 nM	120 ± 20 nM
8	N^6-phenethyladenosine	12.7 nM	161 nM	240 ± 58 nM
9	N^6-dimethyladenosine[6]	10,000 nM	28,900 ± 8,500 nM	32,500 ± 5,100 nM
10	DPMA	142 nM	4.4 nM	3,570 ± 1,700 nM
11	N^6-(2-sulfo)ethyladenosine	41 %	0 %	32,400 ± 7,600 nM
12	N^6-p-sulfophenyladenosine (SPA)	74 nM	8,900 nM	526 ± 142 nM
13	N^6-3-(p-sulfophenyl)propyl adenosine	610 nM	3,840 nM	844 ± 67 nM
14	N^6-4-(p-sulfophenyl)butyl adenosine	432 nM	11,300 nM	808 ± 116 nM
15	1-deaza-2-chloro-N^6-CPA (DCCA)	1.6 nM	13,200 nM	770 ± 234 nM

Table 1: (continued)

#	Compound	A$_1$-affinity[a]	A$_2$-affinity[b]	A$_3$-affinity[c]
16	2-chloroadenosine[10]	9.3 nM	63 nM	1,890 ± 900 nM
17	2-chloro-N^6-CPA	0.6 nM	950 nM	237 ± 71 nM
18	2-phenylaminoadenosine (CV 1808)	560 nM	119 nM	4,390 ± 1,170 nM
19	CGS 21680	2,600 nM	15 nM	584 ± 32 nM
20	5'-carboxamidoadenosine(CA)^{e,6}	72.6 nM	120 nM	1,410 ± 60 nm
21	NMCA^e	83.6 nM	66.8 nM	72 ± 16 nM
22	NECA	6.3 nM	10.3 nM	113 ± 34 nM
23	5'-N-cyclopropyl-CA^{e,10}	6.4 nM	13.4 nM	1,600 ± 70 nM
24	N^6-benzyl-5'-N-cyclopropyl-CA	112 ± 13 nM	55 ± 6 nM	103 ± 22 nM
25	5'-N-aminoethylamino-CA	0 %	12.5 %	14,700 ± 2,540 nM
26	5'-N-boc-aminoethylamino-CA	0 %	0 %	18,000 ± 4,270 nM
27	N^6-cyclohexyl1NECA^7	0.43 nM	170 nM	16.0 ± 5.4 nM
28	N^6-benzyl1NECA	87.3 ± 13.9 nM	95.3 ± 24.6 nM	6.8 ± 2.5 nM
29	N^6-dimethyl1NECA^7	9,600 nM	13,500 ± 3,600 nM	2,260 ± 490 nM
30	N^6-benzyl-N^6-methyladenosine^7	7,600 ± 1,900 nM	40,100 ± 6,200 nM	78.4 ± 4.6 %
31	8-bromoadenosine^3	41.5 ± 3.2 %	22,700 ± 5,100 nM	31.3 ± 6.0 %
32	3-deazaadenosine^6	21,500 nM	59,800 ± 4,600 nM	61,700 ± 34,500 nM
33	7-deazaadenosine (tubercidine)^6	> 100,000 nM	48.3 ± 0.4 %	38.9 ± 17.7 %

Table 1: (continued)

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
34	adenosine-N^1-oxide[3]	246 ± 31 nM	328 ± 60 nM	3,090 ± 1,910 nM
35	NECA-N^1-oxide	154 ± 20 nM	101 ± 19 nM	468 ± 58 nM
36	N^6-benzyladenosine-N^1-oxide	864 ± 88 nM	8,530 ± 1,250 nM	7,250 ± 1,680 nM
37	N'-(4-nitrobenzyl)NECA	341 ± 52 nM	190 ± 52 nM	60 ± 3 nM
38	N'-(2-nitrobenzyl)NECA	196 ± 52 nM	83.5 ± 9.5 nM	27 ± 5 nM
39	N^6-benzyl-5'-carboxamidoadenosine	580 ± 99 nM	423 ± 46 nM	246 ± 35 nM
40	N^6-benzyl-NMCA	898 ± 124 nM	597 ± 42 nM	16 ± 1 nM
41	N^6-(3-bromobenzyl)-NMCA	65 ± 2 nM	64 ± 9 nM	1.0 ± 0.3 nM
42	N^6-(3-iodobenzyl)-NMCA (3-IB-MECA)	54 ± 5 nM	56 ± 8 nM	1.1 ± 0.3 nM
43	N^6-(3-nitrobenzyl)-NMCA	735 ± 5 nM	441 ± 45 nM	19 ± 1 nM
44	N^6-(3-aminobenzyl)-NMCA	1,000 ± 60 nM	794 ± 118 nM	28 ± 13 nM
45	N^6-(3-acetamidobenzyl)-NMCA	7,370 ± 690 nM	12.5 ± 1.9 %	65 ± 15 nM
46	N^6-(3-methylbenzyl)-NMCA	322 ± 23 nM	415 ± 33 nM	10 ± 3 nM
47	N^6-(3-trifluoromethylbenzyl)-NMCA	496 ± 50 nM	574 ± 49 nM	31 ± 6 nM
48	N^6-(4-chlorobenzyl)-NMCA	478 ± 38 nM	2,730 ± 210 nM	17 ± 10 nM
49	N^6-(4-bromobenzyl)-NMCA	516 ± 38 nM	2,460 ± 380 nM	12 ± 5 nM
50	N^6-(4-aminobenzyl)-NMCA	431 ± 45 nM	1,590 ± 180 nM	14 ± 3 nM

Table 1: (continued)

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
51	N^6-(3-iodo-4-aminobenzyl)-NMCA (AB-MECA)	18 ± 5 nM	316 ± 43 nM	1.27 ± 0.18 nM
52	N^6-(3-sulfobenzyl)-NMCA triethylammonium salt	> 10,000 nM	> 10,000 nM	1,310 ± 110 nM
53	N^6-(4-sulfobenzyl)-NMCA triethylammonium salt	0 %	0 %	4,500 ± 480 nM
54	N^6-benzyl-NECA	87 nM	95 nM	6.8 nM
55	N^6-(R-phenylethyl)-NECA	3.2 ± 0.1 nM	259 ± 15 nM	18 ± 4 nM
56	N^6-(S-phenylethyl)-NECA	65 ± 4 nM	1,650 ± 120 nM	494 ± 91 nM
57	N^6-(3-fluorobenzyl)-NECA	51 ± 7 nM	32 ± 4 nM	10.7 ± 1.2 nM
58	N^6-(3-chlorobenzyl)-NECA	22 ± 3 nM	19.0 ± 0.3 nM	1.1 ± 0.3 nM
59	N^6-(3-bromobenzyl)-NECA	10.9 ± 1.5 nM	6.2 ± 0.7 nM	2.8 ± 1.9 nM
60	N^6-(3-iodobenzyl)-NECA	7.7 ± 0.9 nM	7.2 ± 0.6 nM	0.88 ± 0.21 nM
61	N^6-(2-nitrobenzyl)-NECA	31 ± 4 nM	24 ± 3 nM	2.8 ± 0.5 nM
62	N^6-(3-nitrobenzyl)-NECA	78 ± 10 nM	35 ± 7 nM	8.7 ± 1.2 nM
63	N^6-(4-nitrobenzyl)-NECA	49 ± 9 nM	574 ± 64 nM	9.0 ± 1.3 nM
64	N^6-(3-methylbenzyl)-NECA	36.5 ± 1.2 nM	17.7 ± 1.8 nM	1.2 ± 0.1 nM
65	N^6-(2-methoxybenzyl)-NECA	52 ± 5 nM	21 ± 3 nM	7.1 ± 0.3 nM
66	N^6-(3-methoxybenzyl)-NECA	69 ± 8 nM	38 ± 6 nM	4.3 ± 0.6 nM

Table 1: (continued)

#	Compound	A$_1$-affinity[a]	A$_2$-affinity[b]	A$_3$-affinity[c]
67	N^6-(4-methoxybenzyl)-NECA	209 ± 30 nM	609 ± 34 nM	11 ± 3 nM
	ribose-modified adenosines			
68	β-L-adenosine[6]	29,000 ± 4,700 nM	25.4 ± 1.1 %	9.5 ± 4.2 %
69	α-D-adenosine[3]	350,000 nM	128,000 ± 25,000 nM	14.2 ± 6.5 %
70	2'-deoxyadenosine[3]	30.9 ± 8.0 %	38.9 ± 2.9 %	28.3 ± 2.3 %
71	2'-O-methyladenosine[6]	29.4 ± 7.5 %	49.0 ± 5.0 %	42.9 ± 9.4 %
72	3'-deoxyadenosine (Cordycepin)[3]	5.8 ± 2.8 %	26.3 ± 3.4 %	32.7 ± 2.0 %
73	5'-deoxyadenosine[3]	269 ± 135 nM	596 ± 54 nM	2,830 ± 460 nM
74	5'-deoxy-5'-aminoadenosine[6]	42,700 ± 6,000 nM	38,500 ± 3,800 nM	30.6 ± 2.2 %
75	5'-deoxy-5'-methylthioadenosine[3]	281 nM	1,100 nM	1,420 ± 530 nM
76	5'-deoxy-5''-isobutylthio adenosine[3]	1,140 ± 130 nM	6,890 ± 1,750 nM	3,360 ± 360 nM
77	S-adenosylmethionine[3]	675 ± 87 nM	2,780 ± 250 nM	2,470 ± 450 nM
78	AMP[4]	-[d]	57.5 ± 4.0 %	17.2 ± 6.3 %
79	adenine-β-D-arabinofuranoside[6]	20.2 ± 8.4 %	26.0 ± 8.4 %	23.7 ± 3.8 %
80	β-D-psicofuranosyladenine[6]	36.1 ± 4.9 %	51.5 ± 7.4 %	21.1 ± 0.9 %
	non-adenosine nucleosides			
81	xanthosine[5]	9.1 ± 2.4 %	8.5 ± 2.5 %	23.4 ± 8.8 %
82	uridine[5]	14.3 ± 6.9 %	2.8 ± 5.2 %	18.9 ± 2.8 %

Table 1: (continued)

#	Compound	A$_1$-affinity[a]	A$_2$-affinity[b]	A$_3$-affinity[c]
83	thymidine[5]	23.4 ± 2.5%	1.7 ± 3.4 %	21.3 ± 4.9 %
84	cytidine[5]	18.0 ± 1.2 %	16.0 ± 1.5 %	24.5 ± 10.2 %
85	inosine[1]	16,700 ± 2,900 nM	50,000 ± 12,700 nM	45,100 ± 38,800 nM
86	guanosine[3]	27,800 ± 9,600 nM	8,500 ± 15,700 nM	98,500 ± 28,700 nM
87	(4-nitrobenzyl)-6-thioguanosine	15,000 ± 3,500 nM	48,500 ± 11,300 nM	40,700 ± 26,300 nM
88	6-thioguanosine[3]	44.2 ± 2.3 %	27.7 ± 5.8 %	44.8 ± 18.1 %
89	6-thiopurine riboside[3]	61.2 ± 3.9 %	33.6 ± 3.3 %	41.9 ± 5.0 %
90	NMCI	0 %	6.2 ± 4.2 %	6,220 ± 1,220 nM
91	NECI	43.7 ± 10.3 %	30.6 ± 2.3 %	5,000 ± 1,150 nM
	non-xanthine adenosine antagonists			
92	CP 66713[8]	270 nM	21 nM	29.7 ± 7.8 %
93	CGS 15943[9]	21 nM	3.3 nM	38.0 ± 14.5 %
94	IQA	1,600 nM	1,400 nM	32.6 ± 10.8 %
95	9-ethyl-N[6]cyclopentyladenine[7]	440 nM	17,000 nM	30.4 ± 9.1 %
96	EHNA	455 ± 10 nM	59.6 ± 2.8 %	57.5 ± 14.3 %
97	amiloride	11,000 nM	17,000 nM	22.0 ± 3.5 %
	simple alkylxanthines			
98	xanthine (X)[1]	298,000 nM	16.2 ± 2.6 %	14.0 ± 7.9 %

Table 1: (continued)

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
99	1-methylX	11,400 nM	36,200 nM	11.1 ± 1.6 %
100	3-methylX	35,000 nM	38.0 ± 0.9 %	18.1 ± 6.7 %
101	7-methylX	52.3 ± 7.9 %	37.7 ± 4.9 %	16.4 ± 9.6 %
102	9-methylX	26.6 ± 3.2 %	16.1 ± 1.9 %	22.8 ± 9.5 %
103	1,3-dimethylX (theophylline, T)	8,500 nM	25,000 nM	23.1 ± 9.5 %
104	1,7-dimethylX (paraxanthine)	30,000 nM	19,400 ± 3,500 nM	15.5 ± 12.1 %
105	1,9-dimethylX[2]	29.4 ± 1.6 %	6.0 ± 6.3 %	17.0 ± 7.9 %
106	3,7-dimethylX (theobromine)[3]	83,400 nM	187,000 nM	19.9 ± 7.1 %
107	3,9-dimethylX[2]	19.7 ± 7.9 %	4.2 ± 5.9 %	19.0 ± 6.8 %
108	1-methyl-3-isobutyl (IBMX)	7,000 nM	16,000 nM	30.1 ± 12.4 %
109	1,3-dibutylX[6]	500 nM	2,930 ± 700 nM	143,000 ± 29,000 nM
110	1,3-dihexylX[6]	1,260 ± 90 nM	14.3 ± 3.0 % (10 μm)	9.2 ± 6.5 % (10 μM)
111	1,3-dibenzylX[6]	2,000 nM	3.61 ± 0.94 % (10 μM)	20.3 ± 8.5 % (10 μM)
112	1,3,7-trimethylX (caffeine, C)	29,000 nM	48,000 nM	30.1 ± 12.4 %
113	1,3,9-trimethylX (isoC)	>100,000,000 nM	14.4 ± 5.7 %	13.2 ± 12.4 %
114	2-thio-3-propylX[6]	26,100 ± 1,500 nM	32,500 ± 4,800 nM	27.7 ± 11.3 %

Table 1: (continued)

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
	7-substituted alkylxanthines			
115	7-benzylT[6]	6,000 nM	46,000	29.7 ± 0.2 %
116	7-β-hydroxyethylT	105,000 nM	17,400 ± 900 nM	21.1 ± 13.3 %
117	T-7-riboside	27,000 ± 3,200 nM	n.t.	89,400 ± 13,400 nM
118	1,3-dipropylX-7-riboside (DPXR)	15,900 ± 1,800 nM	32.0 ± 1.1 %	81,200 ± 10,700 nM
119	1,3-dibutylX-7-riboside (DBXR)	4,190 ± 1,030 nM	19,500 ± 4,200 nM	6,030 ± 2,320 nM
120	1-benzyl-3-butyl-7-(1-β-D-ribofuranosyl)X	2,190 ± 390	3,800 ± 2,200	12,900 ± 300
121	1-butyl-3-benzyl-7-(1-β-D-ribofuranosyl)X	1,720 ± 30	4,510 ± 1,230	12,400 ± 20
122	1,3-dibutyl-7-(5-methoxycarbonyl-β-D-ribofuranosyl)X	n.t.	n.t.	3,230 ± 590
123	1,3-dibutyl-7-(1-β-D-ribofuranuronamido)X	2,940 ± 350	59,200 ± 5,200	15,300 ± 700
124	1,3-dibutyl-7-(1-β-D-ribofuranosyl)X-5'-methyluronamide	30%	39,200	229 ± 27
125	1,3-dibutyl-7-(1-β-D-ribofuranosyl)X-5'-dimethyluronamide	n.t.	n.t.	228,000 ± 9,000

Table 1: (continued)

#	Compound	A_1-affinity[a]	A_2-affinity[b]	A_3-affinity[c]
126	1,3-dibutyl-7-(1-β-D-ribofuranosyl)X-5'-ethyluronamide	7,500	17,000	602 ± 76
	8-substituted alkylxanthines			
127	8-phenylT	86 nM	850 nM	12.0 ± 6.0 %
128	8-cyclopentylT	11 nM	1,400 nM	38.7 ± 2.5 %
129	8-cyclopentyl-1-propylX[6]	226 ± 37 nM	48,700 ± 5,000 nM	22.6 ± 7.7 %
130	8-cyclopentyl-1,3-dipropylX (CPX)	0.46 nM	340 nM	18.7 ± 2.9 % (10 μM)
131	8-cyclohexylC[6]	28,000 nM	10,400 ± 2,600 nM	35.2 ± 1.8 %
132	8-chloroT[1]	30.2 ± 6.7 %	24.7 ± 3.9 %	16.8 ± 9.5 %
133	XAC	1.2 nM	63 nM	7.1 ± 0.9 %
134	CSC	28,200 nM	54 nM	4.2 ± 5.1 % (10 μM)
135	8-sulfophenyl-1,3-dipropylX	140 nM	790 nM	21.9 ± 6.2 %

[a] Displacement of [³H]PIA (or [³H]CHA) binding from rat brain membranes.

[b] Displacement of [³H]CGS 21680 (or [³H]NECA in the presence of 50 nM CPA) from rat striatal membranes.

[c] Displacement of [¹²⁵I]APNEA or [¹²⁵I]AB-MECA binding from membranes of CHO cells stably transfected with the rat A_3-cDNA.

75

Table: (continued)

d AMP displayed an extremely high slope factor (3.62 $\pm$ 0.39 μM) in A_1 displacement. The apparent K_i was 47.5 $\pm$ 6.5 μM.

e Values at A_1 and A_2 receptors are taken from Bruns et al., Mol. Pharmacol., 29, 331-346 (1986).

1 Aldrich, Milwaukee, WI

2 Fluka, Ronkonkoma, NY

3 Sigma, St. Louis, MO

4 Boehringer Mannheim, Indianapolis, IN

5 Janssen/Spectrum, Gardena, CA

6 Dr. J. Daly, National Institutes of Health, Bethesda, MD

7 Dr. R. Olsson, University of South Florida, Tampa, FL

8 Dr. R. Sarges, Pfizer, Groton, CT

9 Dr. J. Francis, Ciba-Geigy, Summit, NJ

10 Research Biochemicals International, Natick, MA

The affinity of adenosine, itself, cannot be accurately determined in this binding assay, due to the presence of adenosine deaminase, which is required to degrade endogenously generated adenosine. Hence, it is

5   not possible to directly compare the affinities of adenosine with the derivatives tested here. The affinity of adenosine has previously been estimated at 30 μM (Zhou et al., supra), but this value should only be taken as a rough approximation.

10   The most potent compounds at the A_3 receptor are N^6-substituted and/or 5'-uronamide substituted adenosine derivatives. In a series of N^6-aryl(alkyl) substituted compounds, N^6-benzyladenosine is more potent (K_i 120 nM) than N^6-phenyladenosine (K_i 802 nM) or N^6-

15 phenethyladenosine (K_i 240 nM) but essentially nonselective. Introduction of a p-sulfo group in N^6-phenyladenosine slightly enhances affinity (SPA, K_i 526 nM) for the A_3 receptor. Two other N^6-sulfo derivatives, N^6-3-(4-sulfophenyl)propyladenosine and N^6-4-(4-sulfo-

20 phenyl)butyladenosine, have affinities in the same range (see Table, supra), but the 2-sulfoethyl derivative, which has a shorter N^6-substituent, is considerably less potent (K_i 32.4 μM). The polar sulfo group is apparently tolerated at A_3 receptors, and sulfo substitution appears

25 to shift affinity in the direction of A_3 selectivity. The 5'-N-methylcarboxamide analogue of N^6-benzyl-adenosine was 37-56 fold selective for A_3 receptors over A_2 and A_1 receptors, respectively, whereas the 5'-N-cyclopropyl-carboxamide analogue was not selective and

30 much less potent. Although the 5'-N-methyl substitution was generally favored over the 5'-N-ethyl substitution, there were several exceptions, such as the 3-nitro and 3-methyl analogues, in which the potency was more favorable in the N-ethyl series. The N^6-cycloalkyl derivatives,

35 CHA and CPA, are among the more potent compounds at A_3 (K_i 167 and 240 nM, respectively), as is the N^6-functionalized congener ADAC (K_i 281 nM). The N^6-

substituted compound, DPMA, has moderate potency at A_3 (K_i 3.57 μM). The affinity of N^6-dimethyladenosine is quite low (K_i 32.5 μM). The affinity of N^6-benzyl-N^6-methyladenosine and N^6-dimethyl-NECA is also considerably

5   lower than the parent compounds N^6-benzyladenosine and NECA. Thus, although disubstitution at N^6 reduces affinity, it enhances selectivity for A_3 receptors (e.g., N^6-dimethyl-NECA is 4-fold selective for A_3 over A_1 and 6-fold for A_3 over A_2). NECA (5'-N-ethylcarboxamide

10   adenosine) is relatively potent (K_i 113 nM). The effects of N^6- and C5'-substitutions appear to reinforce each other. Thus, the 5'-N-ethylcarboxamide of CHA is considerably more potent than either CHA (K_i 167 nM, 10-fold) or NECA (K_i 113 nM, 7-fold), and, with a K_i of 16

15   nM, it is a highly potent compound at A_3 receptors. Likewise, N^6-dimethyl-NECA is 14-fold more potent than N^6-dimethyladenosine at A_3 receptors. N^6-benzyl-NECA has a high A_3 affinity, with a K_i value of 6.8 nM, and it is 18-fold more potent than the parent compound N^6-

20   benzyladenosine at A_3 receptors. N^6-benzyl NECA is also A_3 selective (13-fold selective for A_3 over A_1 and 14-fold selective for A_3 over A_2).

       Comparisons of benzyl group substitutions were made for both the N-ethyl and the N-methyluronamide series.

25   The R-isomer of N^6-phenylisopropyl-adenosine is favored over the S-isomer. For the doubly modified N^6-(1-phenylethyl)-5'-uronamido-adenosine analogues, the R-configuration is also favored by a factor of 27 at A_3 receptors. The presence of a sulfo group on the benzyl

30   substituent (Ijzerman et al., Drug Design Disc., 9, 49-68 (1992)) is expected to result in selectivity for peripheral versus central A_3 receptors in vivo (see compounds 52 and 53 in Table 1).

       Among N^6-benzyl-5'-N-ethyluronamides, selectivity

35   for A_3 vs. A_1-receptors ranged from 4- to 30-fold and was greatest for the 3-methyl derivative. 3-Chloro-, 3-methoxy-, and 3-nitro- derivatives were also very

selective for A_3 over A_1 receptors. Selectivity for A_3 over A_{2a} receptors ranged from 2- to 64-fold and was greatest for the 4-nitro derivative and the 4-methoxy derivative. Substitution at the 4 position provided high selectivity for A_3 over A_{2a} receptors in both the N-methyl and N-ethyl series. Substitution at the 3-position generally favored A_3-potency and selectivity. 3-Halo benzyl derivatives were particularly potent. The 3- and 4-bromo derivatives in the 5'-N-methyl series, respectively, were both relatively selective, but the affinity at A_3 receptors was 6-fold greater with substitution at the 3-position than at the 4-position. In the 5'-N-ethyl series, the order of potency at A_1 and A_{2a} receptors was I ~ Br > Cl > F. At A_3 receptors, the order was I ~ Cl > Br > F. In the 5'-N-ethyl series, the 3-iodobenzyl analogue and to a lesser degree the 3-bromo analogue were very highly potent and selective for A_3 receptors. 5'-N-Methyl-N^6-(3-iodobenzyl)adenosine displayed a K_i value of 1.0 nM at A_3 receptors and selectivity over A_1 and A_{2a} receptors of 49 and 51-fold, respectively.

The 3-methyl and 3-trifluoromethyl analogues were prepared in the N-methyl series. The trifluoromethyl analogue was weaker at A_3 receptors. Thus, an electron withdrawing group at the 3-position is not favorable for potency, further supported by the relatively modest potency of the 3-nitro derivative.

The acetamido derivative demonstrated that there is an unfavorable interaction at this site on the receptor. The 3-acetamido group is not favored at A_1 and A_3 receptors and entirely disallowed at A_{2a} receptors. Thus, N^6-(3-acetamidobenzyl)-NMCA is 110-fold and >500-fold selective for A_3 over A_1 and A_{2a} receptors, respectively.

N^6-(4-amino-3-iodobenzyl)-NMCA weakly displaced radioligand from A_3 but not A_1 or A_{2a} receptors; thus, it is a highly selective ligand. It is evident that a

79

negative charge at the 4-benzyl position is poorly
tolerated at all three receptor subtypes.

The 8 position of the purine moiety is not amenable
to substitution in A_3 receptor binding. The 2-position
5   may be substituted without eliminating recognition at A_3
receptors; however, among the few analogues examined
there was no indication of enhancement of A_3 selectivity.

Modification of the N-1 position (either oxidation
to the N-oxide or 1-deaza analogues) is tolerated at A_3
10 receptors. The 5'-N-ethyl uronamide modification was
combined with various changes at the N-1 position. NECA-
N-1-oxide was not selective but the loss of potency
versus NECA was greater for A_1 and A_{2a} receptors than for
A_3 receptors. Introduction of larger groups, such as 2-
15 and 4-nitrobenzyl, into NECA did not affect potency at A_1
and A_{2a} receptors but increased potency at A_3 receptors by
an order of magnitude.

Certain C2-modifications are tolerated to a degree
at the A_3 receptor. 2-chloroadenosine and 2-phenylamino-
20 adenosine are of intermediate potency (K_i 1.9 and 4.4 μM,
respectively). An N^6-substituted derivative (2-chloro-N^6-
CPA, K_i 237 nM), and one bearing a 5'-N-ethylcarboxamide
group (CGS 21680, K_i 584 nM), are more potent C2-
substituted derivatives.

25 With regard to modifications of the ribose moiety,
both the L-enantiomer and the α-anomer of adenosine are
virtually inactive (IC_{50} >> 100 μM).
Psicofuranosyladenine, which contains an extra CH_2OH-
group at C1', is also very weak. 2'-Deoxy-, 2'-O-methyl
30 and 3'-deoxyadenosine all have low affinity (IC_{50} > 100
μM), and inversion of the stereochemistry of the 2'-OH
group (adenine-β-D-arabinofuranoside) similarly results
in a low affinity compound. Thus, the presence of the
2'-OH in the S-configuration and the 3'-OH appear to be
35 essential for high affinity. The 5'-position is more
amenable to modifications than the 2'- or the 3'-
position. 5'-Deoxy, 5'-thioether and 5'-uronamide

80

substitutions are tolerated at A_3 receptors. The 5'-
deoxy derivative of adenosine is moderately potent (Ki
2.83 μM), and, as previously indicated, the 5'-N-
ethylcarboxamide derivative (NECA) is one of the more
5   potent compounds tested (K_i 113 nM). Although it is an
A_3 agonist, it is not A_3 selective -- favoring A_1 and A_{2a}
receptors over A_3 receptors by an order of magnitude in
affinity. The primary carboxamide (CA) is weaker than
NECA at A_3 receptors with roughly the same selectivity
10   ratios. NMCA was the most favored 5' uronamide in
binding to A_3 receptors but was nonselective, although
moderately potent at A_3 receptors. 5'-N-Aminoethylamino-
CA and 5'-N-boc-aminoethylamino-CA were much less potent
than NECA in receptor affinity but were A_3-selective.
15   Some 5'-deoxyadenosine derivatives, including those with
methylthio-, isobutylthio- and methionine substituents
have affinities in the lower micromolar range, whereas
5'-deoxy-5'-aminoadenosine and AMP (bearing a 5'-
phosphate group) are virtually inactive ($IC_{50} > 100$ μM).
20   These results suggest that the ribose domain of the A_3
receptor may be quite similar to the ribose domains of
the A_1 and A_2 receptors.
        6-Thiopurine riboside and 8-bromoadenosine both have
low affinity ($IC_{50} > 100$ μM) at A_3. A bulky 8-substituent
25   forces the ribose moiety into a predominantly syn
conformation, which is a likely explanation for its
inactivity. 7-Deazaadenosine has a $IC_{50} \gg 100$ μM, which
indicates the importance of N7. 3-Deazaadenosine is
slightly more potent, with a K_i of 62 μM. 1-Deaza-2-
30   chloro-CPA (DCCA, K_i 770 nM) is three-fold less potent
than 2-chloro-CPA (K_i 237 nM). This suggests that the
presence of N1 is not crucial, i.e., if the nitrogen is
replaced with carbon, for example, the compound will
still react with the A_3 receptor. The N^1-oxides of
35   adenosine, NECA and N^6-benzyladenosine, are moderately
potent compounds (K_i 3.09, 0.47 and 7.25 μM,
respectively).

Of the unsubstituted non-adenine nucleosides tested,
only inosine (K_i 45 μM) and guanosine (K_i 99 μM) show some
affinity for the A_3 receptor. The 5'-N-ethylcarboxamide
derivative of inosine (NECI) is more potent (K_i 5 μM),
5   which is consistent with the affinity-enhancing effect of
the 5'-carboxamido substituent of NECA. The 5'-N-methyl-
carboxamide derivative of inosine (NMCI) has
approximately the same affinity as NECI but apparently
has increased selectivity. NECI is also selective for A_3
10   receptors, having an IC_{50} larger than 100 μM at A_1 and A_2
receptors. An adenosine transport inhibitor, (4-
nitrobenzyl)-6-thioguanosine (K_i 41 μM), is slightly more
potent than the parent compound, guanosine.

Of the non-xanthines tested, CGS 15943, CP 66713,
15   1H-imidazo[4,5-c]quinolinamine, 9-ethylcyclopentyladenine
and amiloride did not appear to be particularly potent,
with K_i-values in the range of 100 μM or larger. The
adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-
nonyl)adenine (EHNA), was somewhat more potent with an
20   IC_{50} slightly better than 100 μM (57.5% displacement at
100 μM).

Xanthine was found to be a very weak displacer of
[125I]APNEA binding (14% at 100 μM). Substitutions at the
1- and the 3-positions enhanced affinity. Compared to
25   theophylline (1,3-dimethylxanthine, 23.1% at 100 μM),
1,3-dibutylxanthine is more potent (K_i 143 μM). 1,3-
Dihexylxanthine (9.2% at 10 μM) and 1,3-dibenzylxanthine
(20.3% at 10 μM) also seem more potent than theophylline,
but their limited solubility precludes direct comparison.
30   Still, even the most potent of these 1,3-substituted
xanthines is quite weak at A_3 receptors.

The 8-substituents do not appear to contribute much
to affinity, and none of the 8-substituted xanthine
derivatives tested is particularly potent (see Table,
35   supra). Substitutions at the 7-position appear to be
tolerated; both caffeine and 7-benzyltheophylline are

82

slightly more potent at A$_3$ receptors than the 7-unsubstituted parent compound, theophylline.

Example 36

5   This example describes the distribution of A$_3$ receptors in mouse brain.

Twenty white male mice were killed by cervical fracture. Brains were rapidly removed, placed in ice-cold 50 mM Tris-HCl buffer (pH 7.4), and cortex,

10   cerebellum, hippocampus, brain stem and striatum were dissected. Tissue was homogenized in 20 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) using a Polytron (setting #6) for 10 seconds. The homogenates were centrifuged at 35000 x g for 15 min at 4°C. The pellets then were

15   resuspended in fresh volume of the same buffer, homogenized with the Polytron and re-centrifuged. The final pellet was stored at -70 °C before use in the receptor binding assay. For the binding assay, membranes were diluted at a protein concentration of 1-3 mg/ml.

20   Protein concentrations were determined by the BCA protein assay reagents (Pierce Chemical Co., Rockford, IL) using bovine albumin as a standard.

[^{125}I]AB-MECA binding to A$_3$ adenosine receptor in mouse brain membranes was performed in 50 mM Tris, 10 mM

25   MgCl$_2$, 1 mM EDTA buffer (pH 7.4) containing 100 μl membrane suspension with ADA (3 units/ml) added. Where applicable, the A$_1$ component of binding was eliminated by the addition of 100 nM CPX (Research Biochemicals International, Natick, MA) to the medium. The final

30   concentration of [^{125}I]AB-MECA ranged from 0.1-4 nM, while the final volume of the preparation was 0.5 ml. Incubations were carried out in duplicate for 90 min at 25°C. Nonspecific binding was defined in the presence of 40 μM R-PIA (N^6-R-phenylisopropyladenosine) and

35   constituted approximately 30% of the total binding. Binding reactions were terminated by filtration through Whatman GF/B filters using a Brandel M24R cell harvester

83

(Brandel Gaithersburg, MD). Filters were washed three
times with 3 ml ice-cold buffer and placed in vials.
Radioactivity was determined in a Beckman 5500B gamma-
counter.

5   Figure 1 is a graph of A_3 adenosine receptor binding
versus concentration of [^{125}I]AB-MECA (nM), which shows a
representative saturation curve for binding at A_3
adenosine receptors in mouse cerebellar membranes using
[^{125}I]AB-MECA in the presence of 100 nM CPX. The K_d value
10   for binding to A_3 receptors was 1.39 ± 0.04 nM with a B_{max}
of 14.8 ± 2.1 fmol/mg protein.

          Linear fitting of Scatchard plots and the saturation
experiments, analyzed by non-linear regression using
computer program GraphPad InPlot (Version 4.0, San Diego,
15   CA), gave similar results for determination of K_d and
B_{max} values. Each experimental result is reported as mean
$\pm$ S.E.M. from three or four experiments. The high
affinity radioligand, [^{125}I]AB-MECA (Ramkumar et al., J.
Biol. Chem., 268, 16887-16890 (1993)), bound specifically
20   to both A_1 and A_3 adenosine receptors in membranes
prepared from regions of NIH Swiss mouse brains.
Specific binding represented ca. 70% of total binding,
with B_{max} values (expressed as fmol specifically bound/mg
protein in parentheses): hippocampus (215 ± 17), cortex
25   (159 ± 15), cerebellum (120 ± 5), and striatum (123 ± 23).
Scatchard analysis indicated K_d values ranging from 1.9
to 2.8 nM. The A_{2a} selective antagonist 8-(3-
chlorostyryl)caffeine (CSC) failed to displace specific
binding of [^{125}I]AB-MECA. The specific binding consisted
30   of two components, since the A_1 selective antagonist 1,3-
dipropyl-8-cycylopentylxanthine (CPX) completely
displaced most but not all of the high affinity [^{125}I]AB-
MECA binding (sigmoidal competition curves had K_i values
ranging from 2 to 8 nM). Thus, the majority of the
35   specific binding of [^{125}I]AB-MECA was to high affinity A_1
receptors. The residual binding, which was not displaced
by CPX at concentrations as high as 1 μM, represented

84

binding to A_3 adenosine receptors. Low levels of A_3
adenosine receptors (18% of total specific binding using
0.4 nM [^{125}I]AB-MECA in the presence of 100 nM CPX) were
detected in the cerebellum and striatum, with even lower

5   levels in the hippocampus and cortex (7-9% of total
specific binding). A Scatchard analysis showed that, in
the presence of 100 nM CPX, the K_d for binding of
[^{125}I]AB-MECA to cerebellar A_3 receptors was 1.39±0.04 nM
with a B_{max} of 14.9±2.1 fmol/mg protein (n = 3).

10   A_3 receptors were found to be present in the mouse
brain, with highest density in the cerebellum and
striatum. Thus, the striatum, which is critical to the
locomotor depressant effects of A_{2a} agonists (Nikodijevic
et al., FEBS Letters, 261, 67-70 (1990)), contains A_3

15   receptors at a density at least 10-fold lower than A_{2a}
receptors. Since previously reported levels of mRNA
coding for A_3 receptors in rat brain indicated the
greatest density in hippocampus and cerebellum (De et
al., Soc. for Neuroscience, 1993, Abstract 42.11), there

20   is either a species difference between rat and mouse, or
the level of message is not entirely predictive of the
level of receptor density. A_1 receptors are localized in
high density in the hippocampus and appear to be involved
in the cerebroprotective effects of adenosine (Schingnitz

25   et al., Nucleosides Nucleotides, 10, 1067-1076 (1991)).
A3 receptors were also detected in the hippocampus but at
densities 1-2 orders of magnitude lower than A_1
receptors.

30   Example 37

This example describes the use of an A_3-selective
adenosine receptor agonist as a locomotor depressant.

Adult male mice (NIH Swiss Strain, 25-30 g) were
housed in groups of ten animals per cage with a light-

35   dark cycle of 12:12 hr. The animals were given free
access to standard pellet food and water and were
acclimatized to laboratory conditions for 24 hr prior to

testing. Each animal was used only once in the activity monitor.

A potent (K_i 1.1 ± 0.3 nM at rat brain A_3 receptors) and selective (50-fold less potent in binding to either
5 A_1 or A_{2a} rat brain receptors) A_3 agonist, 3-IB-MECA, was selected for _in vivo_ studies. The locomotor effects in mice of 3-IB-MECA alone or in combination with potent and selective A_1 and A_{2a} receptor antagonists were examined.

Locomotor activity of individual animals was studied
10 in an open field using a Digiscan activity monitor (Omnitech Electronics Inc., Columbus, OH) equipped with an IBM-compatible computer. The computer-tabulated measurements represent multivariate locomotor analysis with specific measures, such as simultaneous measurements
15 of ambulatory, rearing, stereotypical, and rotational behaviors. Data were collected in the morning, for three consecutive intervals of 10 min each, and analyzed as a group. Statistical analysis was performed using the Student t-test. The results are reported as mean ±
20 standard error for each point. All drugs were dissolved in a vehicle consisting of a 20:80 v/v mixture of Alkamuls EL-620 (Rhone-Poulenc, Cranbury, NJ) and phosphate-buffered saline, except for CSC, which was dissolved initially in DMSO and diluted in at least 20
25 volumes of vehicle. Drugs were administered i.p. in a volume corresponding to 5 ml/kg body weight. Where applicable, the antagonist was injected 10 minutes before the agonist. After injection of agonist, the mouse was placed in the activity monitor for 5 min before data
30 collection was begun.

Figure 2 is a graph of total distance traveled by the mice (cm/30 min) versus dose of the A_3 adenosine receptor agonist 3-IB-MECA (μg/kg), which shows the locomotor activity in mice. The *p value is < 0.05 vs.
35 vehicle control (n = 6-19). 3-IB-MECA administered i.p. in a dose range of 0.01 to 0.3 mg/kg was found to be a locomotor depressant (based on total distance travelled),

86

with a threshold dose of 0.01 mg/kg and maximal
depression reached at about 0.1 mg/kg (Figure 2).
Unlike depression elicited by selective A_1 and A_{2a}
agonists, the depression did not exceed 90% at the higher

5   doses, but reached a plateau at *ca.* 60% below control
level (Figure 2). Vertical activity, stereotype counts,
and rotational movement were depressed in a dose-
dependent manner following administration of 3-IB-MECA;
however, average distance per move and average speed were

10   not changed significantly.

Administration of 3-IB-MECA also caused rapid
scratching behavior, the frequency of which appeared to
increase with the dose of the A_3 agonist. Since
activation of A_3 receptors causes release of histamine in

15   cultured mast cells, it was proposed that the scratching
could be related to histamine. Coadministration of a
histamine H_1-antagonist, cyproheptadine at 10 mg/kg i.p.
eliminated this behavior, while at 1 mg/kg dose of
cyproheptadine the effect was only partial.

20   Figure 3 is a bar graph of total distance
traveled/30 min (percent of control) versus adenosine
receptor agonist, which shows the locomotor activity in
mice following injection of an A_1- (CPA, 100 μg/kg), A_{2a}-
(APEC, 16 μg/kg), or A_3- (3-IB-MECA, 100 μg/kg) selective

25   agonist and the effects of coadministration of selective
antagonists (n = 6-19). Locomotor activity as a percent
of control is shown for no antagonist (unshaded bars) or
for coadministration of selective xanthine antagonists:
CPX (shaded bars, A_1, 0.25 mg/kg) or CSC (hatched bars,

30   A_{2a}, 1.0 mg/kg). The *p value < 0.05 vs. adenosine
agonist alone.

The highly A_1-selective antagonist CPX (0.25 mg/kg)
completely reversed locomotor depression elicited by the
potent A_1 agonist CPA (Research Biochemicals

35   International, Natick, MA) at its determined ED_{50} value of
100 μg/kg i.p (Figure 3). CPX did not diminish the
depressant effects of either the A_{2a}-selective agonist

APEC or 3-IB-MECA at doses chosen to cause comparable *ca.* 50% reduction in locomotor activity.

The xanthine antagonist CSC is highly A_{2a}-selective in binding assays, functional adenylate cyclase assays, and in vivo with respect to locomotor activity. A dose of CSC of 1 mg/kg caused a small, statistically insignificant reversal of the 3-IB-MECA-mediated locomotor depression (Figure 3). The same dose of CSC has been shown to cause a complete reversal of the behavioral depression elicited by APEC in the same experimental model.

The non-reversal of locomotor depression by the A_1- and A_{2a}-adenosine antagonists is consistent with in vitro observations with rat A_3-receptors, ie. the inability of xanthines to antagonize at this site (Zhou et al., PNAS USA, 89, 7432-7436 (1992); van Bergen et al., ACS 206th National Meeting, Chicago, IL, August, 1993, Abstract MEDI217). Both CPX and CSC are known to act centrally (Jacobson et al., FEBS Letters, 323, 141-144 (1993)).

Example 38

This example demonstrates the cerebroprotective effects of chronic administration of an A_3-selective adenosine receptor agonist.

Female Mongolian gerbils were treated with 100 μg/kg IB-MECA (intraperitoneally) either 15 min prior to or for 10 days followed by one drug-free day prior to 10 min or 20 min forebrain arrest of cerebral circulation (ischemia). Ischemia was effected by bilateral occlusion of the carotid arteries. Control gerbils were injected with saline (0.15 ml, i.p. for 10 days). During postischemic recovery, the rate of cerebral blood flow return to pre-ischemia level, neurological status, and survival were monitored. After 7 days, all surviving animals were perfused with formaldehyde for further studies of morphology and histochemistry.

88

Figure 4 is a graph of % survivors versus days after ischemia and sets forth the results of this experiment. As shown in Figure 4, 60% of control gerbils survived for 7 days after 10 min ischemia (O, n=15). A 40% mortality was realized for control gerbils within the initial 36 hr post-ischemia. Ten percent of those gerbils treated with IB-MECA 15 min prior to 10 min ischemia survived for 7 days after 10 min ischemia (□, n=15). A 90% mortality was realized for the acutely treated gerbils within 12 hr post-ischemia. All those gerbils that had been chronically treated with IB-MECA prior to 10 min ischemia survived for 7 days after 10 min ischemia (△, n=15). Twenty percent of control gerbils survived for 7 days after 20 min ischemia (●, n=20), whereas 90% of those gerbils that had been chronically treated with IB-MECA prior to the 20 min ischemia survived for 7 days (▲, n=20). Of the control gerbils exposed to 10 min ischemia, 30% demonstrated seizures, 40% demonstrated mild forelimb paresis, 20% demonstrated ptosis, and 70% demonstrated hyperactivity. Of those gerbils treated with IB-MECA 15 min prior to 10 min ischemia, 60% demonstrated seizures, and 40% demonstrated irreversible coma. In contrast, only 40% of gerbils treated with IB-MECA for 10 days prior to 10 min ischemia demonstrated mild hyperactivity.

Figure 5 is a graph of the rate of cortical blood flow in the gerbils (% change from pre-ischemia) versus time after 10 min cerebral ischemia (min) in the presence and absence of the A_3 adenosine receptor agonist IB-MECA. As shown in Figure 5, full recovery of post-ischemic blood flow was realized in control animals subjected to 10 min ischemia within 2.5 hr (O). One of those gerbils treated with IB-MECA 15 min prior to 10 min ischemia realized full recovery of blood flow in just over 3.5 hr (▲). In contrast, full recovery of blood flow was realized in gerbils treated with IB-MECA for 10 days prior to 10 min ischemia in about 90 min (●). No

neurological side effects were observed in acutely and
chronically treated gerbils that were not subjected to
ischemia.

Figure 6 is a graph of % survivors versus days post-
ischemia in a repeated experiment. Figure 6 shows that
the end-point mortality for control animals was 53% (○);
the end-point mortality for acutely treated animals was
90% (▲, p<0.01). In contrast, 90% of the chronically
treated animals survived (●, p<0.01).

Figure 7 is a bar graph of % surviving neurons in
gerbil hippocampus following 10 min forebrain ischemia
versus treatment method (control (○), acute (15 min, ▲)
or chronic (●)) with 100 μg/kg IB-MECA, wherein n
represents the number of hemispheres in each group
(control: n=8; acute: n=2; chronic: n=16, where p<0.01
vs. control). Acute administration of the drug resulted
in the impairment of postischemic cortical blood flow
rate, increased mortality rate, and extensive neuronal
destruction in the hippocampus. Chronic (10 days)
treatment with IB-MECA prior to 10 min ischemia resulted
in improved post-ischemic blood reflow, increased
survival, and >80% survival of hippocampal neurons,
whereas acute treatment with IB-MECA protected only about
30% of hippocampal neurons.

These data suggest that IP3 is a second messenger of
the A_3 receptor and show that the A_3 receptor is
desensitized by chronic administration of an A_3-selective
adenosine receptor agonist, e.g., IB-MECA.

The above data show that chronic administration of
an A_3-selective adenosine receptor agonist provides
cerebroprotection from the effects of ischemia.

Example 39

This example demonstrates the cardiovascular effects
of an A_3-selective adenosine receptor agonist.

Rats (each weighing 300-340 g) were lightly
anesthetized using 1.5-2% halothane. They were monitored

90

for heart rate with a tail sensor and blood pressure
using the tail cuff method. Peripheral blood flow
(scalp) was monitored using a Perimed laser Doppler blood
flow apparatus, with a narrow diameter probe placed in
5   contact with the shaved skin above the center of the
cranium. A small amount of clear gel was applied to
maintain uniform transmission of light. Body temperature
was measured using a rectal_probe. Respiratory rate was
also recorded. Measurements were made at regular
10   intervals: every 2 min for the first ten min period, and
every five min thereafter, until 30 min, at which time a
30 min interval was used, until 90 min. Each drug group
had 5-7 animals.   Drugs were dissolved in a vehicle
consisting of an 80:20 mixture of Alkamuls EL-620 and
15   saline, pH 7.4. For a 0.1 mg/kg dose, a solution of 2.5
mg/ml in vehicle was prepared. When administered alone,
the agonist was given at the beginning of the monitoring
period. When coadministered with an antagonist, the
antagonist was given at the beginning of the monitoring
20   period, and the agonist was given after 10 min.

As a control experiment, the effects of mild
halothane anesthesia on cardiovascular parameters were
investigated and found to be minimal. A dose of 0.1
mg/kg, i.p., of IB-MECA was chosen, since at this dose
25   locomotor depression in NIH mice, which are insensitive
to either an A_1 or an A_{2a} antagonist, is nearly maximal.
The A_3 agonist administered alone caused a lowering of
the blood pressure with little effect on heart rate. The
effects of this agonist on blood flow and respiratory
30   rates were not significant. In contrast, a potent and
selective A_1 agonist, N^6-cyclopentyladenosine, at a dose
of 0.1 mg/kg, i.p., caused an intense drop in both blood
pressure (by 50%) and heart rate (by 20%) from their
initial values. The hypotensive effect began to wane at
35   approximately 20 min post-injection, but the bradycardiac
effect of the A_1 agonist was prolonged during the entire
monitoring period (90 min).

91

The effect of adenosine agonists on temperature was
also measured (although the temperature of the animal was
externally maintained by a heat source at 37 °C). IB-
MECA maintained the original temperature until about 30
5   min post-injection, after which time the temperature
dropped gradually by approximately 0.5°C. The
hypothermic effects of A_1 agonists have been described.
Busto et al., J. Cereb. Blood Flow Metab., I, 729-738
(1987); Welsh et al., J. Cereb. Blood Flow Metab., 10,
10   557-563 (1990); and Minamisawa et al., Stroke, 21, 758-
764 (1990). The temperature of the animals dropped by
approximately 1 °C over the course of the monitoring
period.

A non-selective adenosine A_1 and A_2 antagonist,
15   BW1433 (1,3-dipropyl-8-(4-carboxyvinylphenyl)xanthine,
Dr. S. Daluge, Burroughs Wellcome, Research Triangle,
NC), was used to eliminate any non-A_3 receptor component
of the biological activity. This agent is particularly
useful for probing the peripheral actions of adenosine
20   (Jacobson et al., J. Med. Chem., 35, 4143-4149 (1992)),
since it does not readily cross the blood-brain barrier.
The dose of 4 mg/kg was selected based on previous
experiments on reversing the antilipolytic effects of
adenosine agonists. This antagonist, a 1,3-
25   dipropylxanthine derivative, alone had biological effects
in vivo. These included a prolonged rise in both blood
pressure and heart rate (to > 40% of initial values in
each case, from 10-30 min post-injection). Blood flow
and respiratory rates were increased by administration of
30   the adenosine antagonist alone. The effect of BW1433 on
body temperature was somewhat erratic, but there were no
major changes.

When IB-MECA was injected 10 min after the adenosine
antagonist BW1433, it still had an effect on blood
35   pressure and heart rate, which were both restored to
approximate control levels by the A_3 agonist IB-MECA.
This is not interpreted as a simple antagonism, since the

92

level of A_1 and A_{2a} antagonism produced by the previously
administered BW1433 is sufficient to block even high
concentrations of agonists selective for those subtypes
(Jacobson et al., J. Med. Chem., 35, 4143-4149 (1992)).

5   Thus, if IB-MECA acted as an agonist at the A_1 and A_{2a}
receptors, its effect would also have been blocked. This
suggests that A_3 receptors may be involved in maintaining
cardiovascular homeostasis, -in case of challenges to the
system that would otherwise raise the blood pressure and

10   heart rate.   Similarly, the blood flow and respiratory
rate were restored to their initial values by the A_3
agonist.   The coadministration of BW1433 and IB-MECA had
nearly no effect on body temperature.

     IB-MECA was found to be 50-fold selective for A_3

15   receptors versus both A_1 and A_{2a} receptors in rat brain.
The above data suggest that it is also selective in vivo
in a cardiovascular model.   It is noteworthy that the Ki
values for inhibition of radioligand binding by CPA (N^6-
cyclopentyladenosine, Research Biochemicals

20   International, Natick, MA) or IB-MECA at A_1 or A_3
receptors, respectively, are both about 1 nM.
Furthermore, the doses of the two agents used in this
Example were also identical (0.1 mg/kg).

     IB-MECA acts in a manner that is highly atypical

25   with respect to previously characterized adenosine
agonists.   The result is that most cardiovascular
functions are maintained at near control values, even in
the presence of a potent but non-selective (active only
at A_1 and A_2, but not A_3 receptors) adenosine antagonist,

30   BW1433.   When the drug is administered alone, the curious
result is that heart rate, respiration, and peripheral
blood flow (scalp) is nearly constant, but there is a
pronounced hypotensive effect that begins within 2 min
post-injection and is prolonged throughout the entire 90

35   min monitoring period.   This reasonably indicates that
this A_3 agonist has a clinically useful half-life in
vivo.

93

Example 40

This example demonstrates the anxiolytic effects of an A_3-selective adenosine receptor agonist.

C57BL/J mice were chronically treated by injection
5   with 100 μg/kg IB-MECA, an A_1-selective adenosine receptor compound, or vehicle. Unlike control groups, IB-MECA chronically-treated animals demonstrated almost no injection-induced anxiety or stress behavioral responses as measured by aggressive and/or flight
10   responses to handling.

Example 41

This example describes the synthesis of N^6-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine.
15   A mixture of 6-chloropurine riboside (Aldrich Chemical Co., 100 mg, 0.35 mmol), triethylamine (0.146 ml, 1.05 mmol), and 3-iodobenzylamine hydrochloride (103 mg, 0.38 mmol) in ethanol (2 ml) was heated for 18 h at 85 °C in a sealed bottle. After the reaction mixture was
20   concentrated to dryness, the residue was purified on a silica gel column chromatography ($CHCl_3$-MeOH, 10:1) to yield N^6-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine (148 mg, 88%) as a colorless solid: m.p. 172 °C; ^1H NMR (DMSO-d_6) d 3.54 (m, 1 H, H-5'a), 3.67 (m, 1 H, H-5'b),
25   3.96 (d, J = 3.3 Hz, 1 H, H-4'), 4.14 (m, 1 H, H-3'), 4.60 (m, 1 H, H-2'), 4.66 (br s, 2 H, CH_2), 5.16 (d, J = 4.4 Hz, 1 H, exchangeable with D_2O, 3'-OH), 5.34 (br s, 1 H, exchangeable with D_2O, 5'-OH), 5.43 (d, J = 6.1 Hz, 1 H, exchangeable with D_2O, 2'-OH), 5.89 (d, J = 6.0 Hz, 1
30   H, H-1'), 7.11 (pseudo t, J = 8.0 and 7.8 Hz, 1 H, H-5''), 7.36 (d, J = 7.6 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.72 (s, 1 H, H-2''), 8.21 (s, 1 H, H-2 or 8), 8.40 (s, 1 H, H-2 or 8), 8.48 (br s, 1 H, exchangeable with D_2O, N^6H,).

35

94

Example 42

The example describes the synthesis of 2-chloro-N[6]-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine.

A mixture of 2-chloro-N[6]-(3-iodobenzyl)-9-[2,3,5-tri-O-benzoyl-β-D-ribofuranosyl]-adenine (760 mg, 0.916 mmol) and NH_3/MeOH (15 ml) was stirred for 66.5 h at room temperature. After the reaction mixture was concentrated to dryness, the residue was purified on a silica gel column chromatography ($CHCl_3$-MeOH, 20:1) to yield 2-chloro-N[6]-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine (445 mg, 94%) as a foam. ^{1}H NMR (DMSO-d_6) d 3.55 (m, 1 H, H-5'a), 3.65 (m, 1 H, H-5'b), 3.94 (d, J = 3.6 Hz, 1 H, H-4'), 4.12 (m, 1 H, H-3'), 4.51 (q, J = 5.5 Hz, 1 H, H-2'), 4.60 (br d, J = 5.7 Hz, 2 H, CH_2), 5.04 (pseudo t, J = 5.7 and 5.5 Hz, 1 H, exchangeable with D_2O, 5'-OH), 5.19 (d, J = 4.9 Hz, 1 H, exchangeable with D_2O, OH), 5.47 (d, J = 6.0 Hz, 1 H, exchangeable with D_2O, OH), 5.83 (d, J = 5.5 Hz, 1 H, H-1'), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-5''), 7.36 (d, J = 7.5 Hz, 1 H, H-4'' or -6''), 7.60 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.74 (s, 1 H, H-2''), 8.43 (s, 1 H, H-8), 8.94 (br t, J = 6.0 Hz, 1 H, exchangeable with D_2O, NH,).

Example 43

This example describes the synthesis of 2-amino-N[6]-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine.

A mixture of 2-amino-6-chloropurine riboside (purchased from Aldrich Chemical Co., 80 mg, 0.26 mmol), 3-iodobenzylamine hydrochloride (71.5 mg, 0.265 mmol), and triethylamine (0.11 ml, 0.79 mmol) in ethanol (1.6 ml) was heated for 24 h at 80 °C. After the reaction mixture was concentrated to dryness the residue was purified on a silica gel column chromatography ($CHCl_3$-MeOH, 20:1->10:1) to yield 2-amino-N[6]-(3-iodobenzyl)-9-[β-D-ribofuranosyl]-adenine (99 mg, 75%) as a colorless solid: m.p. 152-154 °C; ^{1}H NMR (DMSO-d_6) d 3.52 (m, 1 H, H-5'a), 3.63 (m, 1 H, H-5'b), 3.89 (m, 1 H, H-4'), 4.10

95

(m, 1 H, H-3'), 4.50 (m, 1 H, H-2'), 4.60 (br s, 2 H, CH$_2$), 5.08 (d, J = 4.6 Hz, 1 H, exchangeable with D$_2$O, 3'-OH), 5.35 (m, 2 H, exchangeable with D$_2$O, 5'- and 2'-OH), 5.73 (d, J = 6.2 Hz, 1 H, H-1'), 5.83 (br s, 2 H,
5   exchangeable with D$_2$O, NH$_2$), 7.11 (pseudo t, J = 7.9 and 7.8 Hz, 1 H, H-5''), 7.36 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.70 (s, 1 H, H-2''), 7.94 (s, 1 H, H-8).

10   Example 44
    This example describes the synthesis of 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.
    A mixture of 2-chloro-N^6-(3-iodobenzyl)-9-[5-
15 (methylamido)-2,3-di-O-benzoyl-β-D-ribofuranosyl]-adenine (27 mg, 0.036 mmol) and NH$_3$/MeOH (15 ml) was stirred for 16 h at room temperature. After rotary evaporation of the volatiles, the residue was purified on a silica gel column chromatography (CHCl$_3$_MeOH, 20:1->10:1) to yield 2-
20 chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine (13.4 mg, 68.7%) as a colorless solid: m.p. 206-207 °C; ^{1}H NMR (DMSO-d$_6$) d 2.72 (d, J = 4.3 Hz, 3 H, -NHCH$_3$), 4.17 (br s, 1 H, H-3'), 4.32 (s, 1 H, H-4'), 5.55 (m, 1 H, H-2'), 4.61 (br d, J = 5.5 Hz, 2
25 Hz, CH$_2$), 5.56 (d, J = 6.4 Hz, 1 H, exchangeable with D$_2$O, 2'-OH), 5.72 (d, J = 4.3 Hz, 1 H, exchangeable with D$_2$O, 3'-OH), 5.92 (d, J = 7.2 Hz, 1 H, H-1'), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-5''), 7.36 (d, J = 7.5 Hz, 1 H, H-4'' or -6''), 7.61 (d, J = 7.8 Hz, 1 H, H-4'' or -
30 6''), 7.75 (s, 1 H, H-2''), 8.27 (br d, J = 4.3 Hz, 1 H, exchangeable with D$_2$O, NH), 8.49 (s, 1 H, H-8), 9.02 (br t, J = 6.2 and 5.7 Hz, 1 H, exchangeable with D$_2$O, N^{6}H).

                          Example 45
35   This example describes the synthesis of N^6-(3-iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.

96

A solution of 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine (10 mg, 0.018 mmol) in 2N CH$_3$NH$_2$/THF (1.5 ml) was heated for 3 days at 90 °C. After the reaction mixture was concentrated to dryness, the residue was purified on a preparative TLC (CHCl$_3$-MeOH, 10:1) to yield N^6-(3-iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine (7 mg, 70%) as a colorless solid: m.p. 190 °C; ^{1}H NMR (DMSO-d$_6$) d 2.66 (d, J = 4.7 Hz, 3 H, -NHCH$_3$), 2.76 (d, J = 4.3 Hz, 3 H, -NHCH$_3$), 4.18 (m, 1 H, H-3'), 4.25 (s, 1 H, H-4'), 4.57 (br s, 2 H, CH$_2$), 4.69 (m, 1 H, H-2'), 5.47 (d, J = 6.5 Hz, 1 H, exchangeable with D$_2$O, 2'-OH), 5.59 (d, J = 4.6 Hz, 1 H, exchangeable with D$_2$O, 3'-OH), 5.84 (d, J = 7.2 Hz, 1 H, H-1'), 6.28 (br d, J = 4.4 Hz, exchangeable with D$_2$O, NH), 7.11 (pseudo t, J = 8.0 and 7.8 Hz, 1 H, H-5''), 7.38 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.70 (m, 1 H, exchangeable with D$_2$O, NH), 7.76 (s, 1 H, H-2''), 8.02 (s, 1 H, H-8), 8.05 (br s, 1 H, exchangeable with D$_2$O, NH). High resolution MS calcd. for C$_{19}$H$_{22}$N$_7$O$_4$I$_1$: 539.3362; Found: 540.0867.

Example 46

This example describes the synthesis of N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.

A solution of 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine (15 mg, 0.029 mmol) and sodium thiomethoxide (4.0 mg, 0.057 mmol) in anhydrous ethylene glycol dimethyl ether (2 ml) was heated at 80 °C, under nitrogen atmosphere, for 3 days. After cooling to room temperature, the reaction mixture was neutralized with glacial acetic acid and evaporated to dryness. The residue was purified on a preparative TLC (CH$_2$Cl$_2$-MeOH, 9.5:0.5) to yield N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine (5.6 mg, 36.5%) as a yellow solid: m.p. 179 °C; ^{1}H NMR

(DMSO-d$_6$) d 2.43 (s, 3 H, -SH$_3$), 2.74 (d, J = 4.3Hz, 3 H,
-NH$\underline{CH}_3$), 3.48 (br s, 2 H, 2 x OH), 4.19 (m, 1H, H-3'),
4.31 (s, 1 H, H-4'), 4.62 (br s, 3 H, CH$_2$ & H-2'), 5.87
(d, J = 7.9 Hz, 1 H, H-1'), 7.11 (pseudo t, J = 8.0 and
7.8 Hz, 1 H, H-5"), 7.90 (d, J = 7.9 Hz, 1 H, H-4" or -
6"), 7.58 (d, J = 7.9 Hz, 1 H, H-4" or -6"), 7.76 (s, 1H,
H-2"), 8.24 (br s, 1 H, NH), 8.35 (s, 1 H, h-8), 8.68 (br
s, 1 H, NH). High resolution mass (measured as ppm) in
FAB mode, Calcd for C$_{19}$H$_{21}$IN$_6$O$_4$S: 557.0468; Found:
557.0482.

Example 47

This example describes the synthesis of 1-O-methyl
5-(t-butyldiphenylsilyl)-β-D-ribofuranoside.

To a mixture of methyl β-D-ribofuranoside (Sigma
Chemical Co., 460 mg, 2.8 mmol) and anhydrous methylene
chloride (20 ml) were added triethylamine (0.468 ml, 3.36
mmol), t-butyldiphenylchlorosilane (0.9 ml, 3.46 mmol),
and DMAP (13.7 mg, 0.112 mmol) successively at room
temperature. The reaction mixture was stirred for 18 h
at room temperature under nitrogen. The reaction mixture
was washed with water (20 ml), saturated ammonium
chloride (20 ml), and brine (20 ml), dried over anhydrous
MgSO$_4$, filtered, and concentrated to dryness. The
residue was separated by silica gel column chromatography
(CHCl$_3$-MeOH, 50:1) to yield 1-O-methyl 5-(t-
butyldiphenylsilyl)-β-D-ribofuranoside [R$_f$ = 0.48 (CHCl$_3$-
MeOH, 10:1), 618 mg, 54.8%] as a thick syrup. ^{1}H NMR
(DMSO-d$_6$) d 0.96 (s, 9 H, t-Bu), 3.22 (s, 3 H, -OCH$_3$),
3.61 (dd, J = 11.0 and 5.3 Hz, 1 H), 3.74 (d, J = 4.4 Hz,
1 H), 3.81 (dd, J = 11.0 and 2.7 Hz, 1 H), 3.90 (m, 1 H),
4.00 (m, 1 H), 4.68 (s, 1 H, H-1'), 4.84 (br s, 1 H,
exchangeable with D$_2$O, OH), 5.05 (br s, 1 H, exchangeable
with D$_2$O, OH), 7.45 and 7.67 (m, 10 H, Ph$_2$). Anal. Calcd
for C$_{22}$H$_{30}$O$_5$Si: C, 65.64; H, 7.51; Found: C, 65.73; H,
7.55.

98

Example 48

This example describes the synthesis of 1-O-methyl
5-(t-butyldiphenylsilyl)-2,3-dibenzoyl-β-D-
ribofuranoside.

5   To a solution of 1-O-methyl 5-(t-
butyldiphenylsilyl)-β-D-ribofuranoside (579 mg, 1.44
mmol) in methylene chloride-pyridine (4:1, 12.5 ml) was
added dropwise benzoyl chloride (0.367 ml, 3.16 mmol) at
0 °C. The reaction mixture was stirred for 2.5 h at 0 °C
10   and for 14.5 h at room temperature. Ice was added to
quench the reaction and the mixture was stirred for 1 h.
Methylene chloride (100 ml) was added and two phases were
separated. Organic layer was washed with water,
saturated ammonium chloride, and brine, dried over
15   anhydrous $MgSO_4$, filtered, and concentrated to dryness to
yield 1-O-methyl 5-(t-butyldiphenylsilyl)-2,3-dibenzoyl-
β-D-ribofuranoside in crude form, which was then purified
by silica gel column chromatography (Hx-EtOAc, 5:1->1:1)
to yield 1-O-methyl 5-(t-butyldiphenylsilyl)-2,3-
20   dibenzoyl-β-D-ribofuranoside in more pure form [R_f = 0.75
($CHCl_3$-MeOH, 10:1), 869 mg, 99%] as a thick syrup. ^{1}H NMR
($CDCl_3$) d 1.05 (s, 9 H, t-Bu), 3.42 (s, 3 H, -OCH_3), 3.86
(dd, J = 11.1 and 4.8 Hz, 1 H, H-5a), 3.92 (dd, J = 11.1
and 4.7 Hz, 1 H, H-5b), 4.48 (dd, J = 10.6 and 4.6 Hz, 1
25   H), 5.15 (s, 1 H), 5.63 (d, J = 5.0 Hz, 1 H), 5.82
(pseudo t, J = 5.8 and 5.4 Hz, 1 H), 7,29-8.18 (m, 20 H,
Ar). Anal. Calcd for $C_{36}H_{38}O_7Si_1$: C, 70.79; H, 6.27;
Found: C, 71.01; H, 6.20.

30   ## Example 49

This example describes the synthesis of 1-O-methyl
2,3-dibenzoyl-β-D-ribofuranoside.

A solution of 1-O-methyl 5-(t-butyldiphenylsilyl)-
2,3-dibenzoyl-β-D-ribofuranoside (849 mg, 1.39 mmol) and
35   1.0M tetrabutylammonium fluoride in THF (1.53 ml, 1.53
mmol) was stirred for 2 h at room temperature. After
evaporation of the solvent, the residue was purified by

silica gel column chromatography (Hx-EtOAc, 1:1) to yield
1-O-methyl 2,3-dibenzoyl-β-D-ribofuranoside [R_f = 0.50
(Hx-EtOAc, 1:1), 461 mg, 89%] as thick syrup. ^{1}H NMR
(DMSO-d_6) d 3.38 (s, 3 H, -OCH$_3$), 3.62 (m, 2 H, H-5), 4.37
(q, J = 5.2 Hz, 1 H, H-4), 5.02 (pseudo t, J = 6.0 and
5.3 Hz, 1 H, exchangeable with D$_2$O, 5-OH), 5.18 (s, 1 H,
H-1), 5.45 (m, 1 H, H-2), 5.53 (t, J = 5.2 Hz, 1 H, H-3),
7.42-7.88 (m, 10 H, Ar). Anal. Calcd for $C_{20}H_{20}O_7$: C,
64.51; H, 5.41; Found: C, 64.37; H, 5.47.

Example 50

This example describes the synthesis of 1-O-methyl-
2,3-dibenzoyl-β-D-ribofuronic acid.

A mixture of 1-O-methyl 2,3-dibenzoyl-β-D-
ribofuranoside (374.8 mg, 1.01 mmol), ruthenium (IV)
oxide (10 mg), and sodium periodate (1161 mg, 5.43 mmol)
in CHCl$_3$:CH$_3$CN:H$_2$O (2:2:3, 14 ml) was stirred vigorously
for 2.5 h at room temperature. Chloroform (20 ml) was
added and semisolid was removed by filtration. The two
layers of filtrate were separated and aqueous layer was
extracted with chloroform (2 x 40 ml). Combined organic
layer and extracts were washed with brine, dried over
anhydrous MgSO$_4$, filtered, and concentrated to dryness.
After drying in vacuo overnight, 1-O-methyl-2,3-
dibenzoyl-β-D-ribofuronic acid (340 mg, 89.5%) was
obtained as a thick syrup. ^{1}H NMR (CDCl$_3$) d 3.56 (s, 3 H,
-OCH$_3$), 4.90 (d, J = 6.1 Hz, 1 H, H-4), 5.25 (s, 1 H, H-
1), 5.66 (d, J = 5.0 Hz, 1 H, H-2), 6.00 (pseudo t, J =
5.7 and 5.4 Hz, 1 H, H-3), 7.32-7.99 (m, 10 H, Ar). Anal.
Calcd for $C_{20}H_{18}O_8$_0.63H$_2$O: C, 58.35; H, 4.71; Found: C,
58.36; H, 4.54.

Example 51

This example describes the synthesis of methyl 1-O-
methyl-2,3-dibenzoyl-β-D-ribofuronate.

N-Ethyl-N'-diaminopropyl-carbodiimide (EDAC, 198 mg,
1.04 mmol) was added to a solution of 1-O-methyl-2,3-

100

dibenzoyl-β-D-ribofuronic acid (0.16 g, 0.414 mmol) in
MeOH (3 ml) and the reaction mixture was stirred for 3 h
at room temperature. After the solvent was removed by
rotary evaporation, the residue was dissolved in

5   chloroform (50 ml), washed with water (30 ml) and brine
(30 ml), dried over anhydrous $MgSO_4$, filtered, and
concentrated to dryness. The residue was purified on a
preparative TLC (Hx-EtOAc, 1:1) to yield methyl 1-O-
methyl-2,3-dibenzoyl-β-D-ribofuronate [R_f = 0.77 (Hx-

10   EtOAc, 1:1), 120 mg, 72.3%] as a colorless solid: m.p.
92.2-93.7 °C; ^{1}H NMR ($CDCl_3$) d 3.52 (s, 3 H, 1-OCH_3), 3.82
(2, 3 H, 5-OCH_3), 4.84 (d, J = 6.2 Hz, 1 H, H-4), 5.21
(s, 1 H, H-1), 5.62 (d, J = 4.8 Hz, 1 H, H-2), 6.01
(pseudo t, J = 5.7 and 5.5 Hz, 1 H, H-3), 7.32-7.99 (m,

15   10 H, Ar). Anal. Calcd for $C_{21}H_{20}O_8$: C, 63.00; H, 5.03.
Found: C, 63.05; H, 5.03.

                         Example 52
     This example describes the synthesis of N-methyl 1-

20   O-methyl-2,3-dibenzoyl-β-D-ribofuronamide.
     A mixture of methyl 1-O-methyl-2,3-dibenzoyl-β-D-
ribofuronate (35 mg, 0.087 mmol) and 2.0 M methylamine in
THF (3 ml) was heated for 15 h at 50 °C in a sealed tube.
The volatiles were removed by evaporation and the residue

25   was reacted with benzoyl chloride (0.15 ml, 1.29 mmol) in
methylene chloride-pyridine (2:1, 6 ml) for 3 h at room
temperature. After a workup similar to that for the
compound of Example 48, the residue was separated by
preparative TLC (Hx-EtOAc, 1:1) to yield N-methyl 1-O-

30   methyl-2,3-dibenzoyl-β-D-ribofuronamide [R_f = 0.28 (Hx-
EtOAc, 1:1) or 0.77 ($CHCl_3$-MeOH, 10:1), 25 mg, 72%] as a
syrup. ^{1}H NMR ($CDCl_3$) d 2.92 (d, J = 5.0 Hz, 3 H, -NHCH_3),
3.58 (s, 3 H, 1-OCH_3), 4.82 (d, J = 5.5 Hz, 1 H, H-4),
5.24 (s, 1 H, H-1), 5.60 (m, 1 H, H-2), 5.87 (t, J = 5.1

35   Hz, 1 H, H-3), 6.68 (br m, 1 H, -NH), 7.33-7.99 (m, 10 H,
Ar). Anal. Calcd for $C_{21}H_{21}NO_7$_0.5H_2O: C, 61.76; H, 5.43;
N, 3.43; Found: C, 61.76; H, 5.24; N, 3.41.

Example 53

This example describes the synthesis of N-methyl 1-O-acetyl-2,3-dibenzoyl-α-D-ribofuronamide and N-methyl 1-O-acetyl-2,3-dibenzoyl-β-D-ribofuronamide.

To a solution of N-methyl 1-O-methyl-2,3-dibenzoyl-β-D-ribofuronamide (1.533 g, 3.84 mmol) and acetic anhydride (3.8 ml, 40.3 mmol) in glacial acetic acid (19 ml) was added dropwise c-H_2SO_4 (1.125 ml, 21.1 mmol) and the reaction mixture was stirred for 15 h at room temperature. After water (30 ml) was added slowly, the mixture was extracted with methylene chloride (150 ml x 3), and the organic layer was washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and connectrated to dryness. The residue was purified on a silica gel column chromatography ($CHCl_3$_MeOH, 20:1) to yield a mixture of (a) N-methyl 1-O-acetyl-2,3-dibenzoyl-α-D-ribofuronamide and (b) N-methyl 1-O-acetyl-2,3-dibenzoyl-β-D-ribofuronamide [R_f = 0.71 and 0.76 ($CHCl_3$-MeOH, 20:1), respectively, 0.55 g, 33.5%] as a foam. Analytical samples were separated on a preparative TLC ($CHCl_3$-MeOH, 20:1). ^{1}H NMR ($CDCl_3$). Compound A: d 2.10 (s, 3 H, -OAc), 2.91 (d, J = 4.9 Hz, 3 H, -NHC$\underline{H}_3$), 4.96 (s, 1 H, H-4), 5.45 (pseudo t, J = 5.5 and 5.0 Hz, 1 H, H-2), 6.08 (d, J = 5.9 Hz, 1 H, H-3), 6.71 (d, J = 4.7 Hz, 1 H, H-1), 6.72 (br s, 1 H, -NH), 7.28 (pseudo t, J = 7.8 and 7.7 Hz, 2 H, Ar), 7.48 (q, J = 7.8 Hz, 3 H, Ar), 7.62 (pseuso t, J = 7.7 and 6.9 Hz, 1 H, Ar), 7.79 (d, J = 7.4 Hz, 2 H, Ar), 8.13 (d, J = 7.8 Hz, 2 H, Ar). Compound B: d 2.17 (s, 3 H, -OAc), 2.90 (d, J = 4.9 Hz, 3 H, -NHC$\underline{H}_3$), 4.89 (d, J = 6.2 Hz, 1 H, H-4), 5.73 (d J = 4.9 Hz, 1 H, H-2), 5.96 (t, J = 5.9 Hz, 1 H, H-3), 6.43 (s, 1 H, H-1), 6.50 (br s, 1 H, -NH), 7.37 (pseudo t, J = 7.8 and 7.6 Hz, 4 H, Ar), 7.48-7.58 (m, 2 H, Ar), 7.93 (d, J = 8.1 Hz, 2 H, Ar), 7.98 (d, J = 7.3 Hz, 2 H, Ar). Anal. Calcd for $C_{22}H_{21}N_1O_8$_$0.3H_2O$: C, 61.05; H, 5.03; N, 3.24; Found: C, 61.09; H, 5.05; N, 3.63.

Example 54

This example describes the synthesis of 2-chloro-N^6-(3-iodobenzyl)adenine.

5   A solution of 2,6-dichloropurine (Aldrich Chemical Co., 1 g, 5.3 mmol), 3-iodobenzylamine hydrochloride (1.7 g, 5.8 mmol), and triethylamine (2.2 ml, 15.35 mmol) in ethanol (10 ml) was stirred for 5 days at room temperature. The colorless solid formed was collected by suction, washed with small amount of cold ethanol, and

10   dried to yield 2-chloro-N^6-(3-iodobenzyl)adenine (1.16 g, 60%): m.p. 222-224 °C; mass (EI) 385: ^{1}H NMR (DMSO-d_6 d 4.59 (br s, 2 H, -C$\underline{H}_2$), 7.13 (pseudo t, J = 8.2 and 7.5 Hz, 1 H, Bn), 7.36 (d, J = 7.5 Hz, 1 H, Bn), 7.61 (d, J = 7.5 Hz, 1 H, Bn), 7.74 (s, 1 H, Bn), 8.14 (s, 1 H, H-8),

15   8.76 (br s, 1 H, exchangeable with D_2O, NH), 13.14 (br s, 1 H, exchangeable with D_2O, NH).UV (MeOH) l_{max} 281.7, 257.5, 232.5 nm.

Example 55

20   This example describes the synthesis of 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-2,3-di-O-benzoyl-β-D-ribofuranosyl]-adenine.

A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (165 mg, 0.43 mmol), ammonium sulfate (catalytic amount), and

25   HMDS (15 ml) was refluxed for 4 h under nitrogen to provide the silylated derivative. The clear solution was concentrated to dryness *in vacuo* with exclusion of moisture and the residue was dissolved in dry dichloroethane (6 ml). A solution of N-methyl 1-O-

30   acetyl-2,3-dibenzoyl-α-D-ribofuronamide and N-methyl 1-O-acetyl-2,3-dibenzoyl-β-D-ribofuronamide (141 mg, 0.33 mmol) in dry dichloroethane (6 ml) and TMSOTf (83 ml, 0.43 mmol) was added, and the reaction mixture was stirred for 0.5 h at room temperature and refluxed for 62

35   h under nitrogen. Saturated NaHCO$_3$ (10 ml) was added, and the mixture was stirred for 15 min. Two layers were separated and the aqueous layer was extracted with

methylene chloride (50 ml x 3), washed with brine, dried
over anhydrous MgSO$_4$, filtered, and concentrated to
dryness. The residue was purified on a preparative TLC
(CHCl$_3$-MeOH, 20:1) to yield 2-chloro-N^6-(3-iodobenzyl)-9-
5   [5-(methylamido)-2,3-di-O-benzoyl-β-D-ribofuranosyl]-
adenine [R$_f$ = 0.58 (CHCl$_3$-MeOH, 20:1), 83 mg, 33%] as a
foam. ^{1}H NMR (CDCl$_3$) d 3.10 (d, J = 4.6 Hz, 3 H, -NHC$\underline{H}_3$),
4.79 (br s, 2 H, CH$_2$), 4.97 (s, 1 H, H-4'), 6.08 (m, 1 H,
H-3'), 6.15-6.25 (m, 3 H, H-2', 1', NH), 7.06-8.06 (m, 15
10   H, Ar), 8.52 (br s, 1 H, NH).

Example 56

This example describes the synthesis of 2-chloro-N^6-
(3-iodobenzyl)-9-[2,3,5-tri-O-benzoyl-β-D-ribofuranosyl]-
15   adenine.

A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (0.84
g, 2.18 mmol), ammonium sulfate (catalytic amount), and
HMDS (20 ml) was refluxed for 5 h under nitrogen to
provide the silylated derivative. The clear solution was
20   concentrated to dryness *in vacuo* with exclusion of
moisture and the residue was dissolved in dry
dichloroethane (6 ml). A solution of 1-O-acetyl-2,3,5-
tri-O-benzoyl-β-D-ribofuranoside (Janssen Chimica
Chemical Co., 1 g, 1.98 mmol) in dry dichloroethane (12
25   ml) and TMSOTf (0.42 ml, 2.18 mmol) was added, and the
reaction mixture was stirred for 20 min at room
temperature and refluxed for 14 h under nitrogen. After
a workup similar to that for the compound of Example 55,
the residue was purified on a silica gel column
30   chromatography (Hx-EtOAc, 2:1) to yield 2-chloro-N^6-(3-
iodobenzyl)-9-[2,3,5-tri-O-benzoyl-β-D-ribofuranosyl]-
adenine [R$_f$ = 0.11 (Hx-EtOAc, 3:1), 1.495 g, 91%] as a
colorless foam. ^{1}H NMR (CDCl$_3$) d 4.69-4.92 (m, 5 H, CH$_2$,
H-4', H-5'), 6.15 (m, 3 H, H-2', H-3', NH), 6.45 (d, J =
35   4.3 Hz, 1 H, H-1'), 7.07 (pseudo t, 1 H, Bn), 7.31-8.10
(m, 20 H, Ar).

104

Example 57

This example describes a radioligand binding assay used to study the structure activity relationship (SAR) at the A_3 receptor in a manner similar to that set forth in Example 35.

Adenosine analogues were tested in radioligand binding assays (Olah et al., Mol. Pharmacol., 45, 978-982 (1994); Schwabe et al., Naunyn-Schmiedeberg's Arch. Pharmacol., 313, 179-187 (1980); Jarvis et al., J. Pharmacol. Exp. Therap., 251, 888-893 (1989)) for affinity at rat brain A_1, A_{2a}, and A_3 adenosine receptors. The compounds were assayed as follows: at A_1 receptors in rat cortical membranes using [^{3}H]N^6-R-phenylisopropyladenosine (Schwabe et al., supra); at A_{2a} receptors in rat striatal membranes using [^{3}H]CGS 21680 (Jarvis et al., supra); at A_3 receptors using [^{125}I]AB-MECA (Olah et al., supra) in membranes of CHO cells stably transfected with cDNA for rat brain A_3 receptors (Zhou et al., Proc. Natl. Acad. Sci. U.S.A., 89, 7432-7436 (1992)).

The preparation of rat brain membranes and CHO cell membranes was carried out essentially as described in van Galen et al., Mol. Pharmacol., 45, 1101-1111 (1994), van Bergen et al., ACS 206th National Meeting, Chicago, IL, Abstract MEDI217 (August 1993), Gallo-Rodriguez et al., J. Med. Chem., 37, 636-646 (1994), Olah et al., Mol. Pharmacol., 45, 978-982 (1994). Adenosine deaminase (ADA) was from Boehringer Mannheim (Indianapolis, IN). [^{3}H]R-PIA was from Amersham (Arlington Heights, IL), and [^{3}H]CGS 21680 was from DuPont NEN (Boston, MA). [^{125}I]AB-MECA was prepared as described by Olah et al., supra.

Binding of [^{125}I]AB-MECA to CHO cells stably transfected with the A_3 receptor clone was performed essentially as described in Gallo-Rodriguez et al., supra, and Olah et al., supra. Assays were performed in 50 mM Tris/10 mM MgCl$_2$/1 mM EDTA buffer (adjusted to pH 8.26 at 5 °C) in glass tubes and contained 100 μl of the

membrane suspension, 50 μl of [^{125}I]AB-MECA (final
concentration 0.3 nM), and 50 μl of inhibitor.
Inhibitors were routinely dissolved in DMSO and were then
diluted with buffer. The final DMSO concentrations never
exceeded 1%; this concentration did not influence
[^{125}I]AB-MECA binding. Incubations were carried out in
duplicate for 1 hour at 37 °C, and were terminated by
rapid filtration over Whatman GF/B filters, using a
Brandell cell harvester (Brandell, Gaithersburg, MD).
Tubes were washed three times with 3 mL of buffer.
Radioactivity was determined in a Beckman gamma 5500B g-
counter. Non-specific binding was determined in the
presence of 200 μM NECA. K_i-values were calculated
according to Cheng-Prusoff (Cheng et al., supra),
assuming a K_d for [^{125}I]AB-MECA of 1.48 nM (Fozard et al.,
Br. J. Pharmacol., 109, 3-5 (1993).

Binding of [^{3}H]PIA to A_1 receptors from rat cortical
membranes and of [^{3}H]CGS 21680 to A_2 receptors from rat
striatal membranes was performed as described previously
(Gallo-Rodriguez et al., supra).

Competition for binding of [^{3}H]NBTI was carried out
by a modification of the procedure of Marangos et al., J.
Neurochem., 39, 184-191 (1982). Rat striatal membranes,
prepared as above, were subjected to incubation for 30
min at 23 °C with 0.3 nM [^{3}H]NBTI and varying
concentrations of the nucleoside derivative in Tris
buffer, pH 7.4 in a total of 0.5 ml. For non-specific
binding, 5 μM S-(p-nitrobenzyl)-6-thioguansine (Sigma,
St. Louis, MO) was added, and specific binding was 95% of
total. A K_d value of 0.15 nM was used in the calculation
of K_i values (Marangos et al., supra). Specific binding
was 95% of total.

Adenylate cyclase was assayed in membranes from CHO
cells stably expressing the rat A_3 receptor, prepared as
above, using the previously reported method described in
van Galen et al., Mol. Pharmacol., 45, 1101-1111 (1994),
van Bergen et al., ACS 206th National Meeting, Chicago,

106

IL, Abstract MEDI217 (August 1993). The method involved
the addition of $[a-^{32}P]$ATP to membranes in the presence of
forskolin to stimulate adenylate cyclase and papaverine
as a phosphodiesterase inhibitor. The reaction was
5   terminated by addition of a stop solution containing
20,000 cpm/ml $[^{3}H]$cyclic AMP. The total radiolabeled
cyclic AMP was isolated on columns of Dowex 50 ion
exchange resin and alumina. Maximal inhibition of
adenylate cyclase activity corresponded to ~40% of total
10   activity under conditions of stimulation (typically by 6-
8 fold) in the presence of 1 μM forskolin.

Table 2: Affinities of 5'-uronamide derivatives in radioligand binding assays at rat brain A_1, A_2, and A_3 receptors[a-c]

Compound	$K_i(A_1)$	$K_i(A_2)$	$K_i(A_3)$	A_1/A_3	A_2/A_3
IB-MECA[d]	54	56	1.1	49	51
[125I]AB-MECA[d]	18	197	1.3	14	160
APNEA	14[f]	172 ± 50	116 ± 18	0.16	1.5
[125I]APNEA	2.1[f]	---	15.5[g]	0.14	---
CGS 21680[e]	2600	15	584	4.4	0.026
APEC	400	5.7	50 ± 24	8	0.11
2-chloro-adenosine[e]	9.3	63	1890	0.0049	0.033
N^6-cyclopentyladenosine[d]	0.59	462	240	0.0025	1.9
2-chloro-N^6-cyclopentyladenosine[d]	0.6	950	237	0.0025	4.0
N^6-(3-iodobenzyl)-adenosine [Example 41]	20.0 ± 8.5	17.5 ± 0.5	9.5 ± 1.4	2.1	1.8
2-chloro-N^6-(3-iodobenzyl)-adenosine [Example 42]	18.5 ± 4.7	38.5 ± 2.0	1.41 ± 0.17	13	27
2-amino-N^6-(3-iodobenzyl)-adenosine [Example 43]	63.8 ± 15.1	117 ± 15	181 ± 30	0.35	0.65
2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine [Example 44]	820 ± 570	470 ± 365	0.23 ± 0.11	3600	2000
N^6-(3-iodobenzyl)-2-methylamino-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine [Example 45]	4890 ± 2580	4120 ± 210	3.12 ± 0.64	1600	1300
N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine [Example 46]	2140 ± 100	3210 ± 1360	2.30 ± 0.96	930	1400

Table 2: (continued)

[a] Displacement of specific [³H]PIA binding, unless noted, in rat brain membranes, expressed as $K_i \pm$ S.E.M. in nM (n = 3-6).

[b] Displacement of specific [³H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as $K_i \pm$ S.E.M. in nM (n = 3-6).

[c] Displacement of specific binding of [¹²⁵I]4-amino-3-iodobenzyladenosine-5'-N-methyluronamide from membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as $K_i \pm$ S.E.M. in nM (n = 3-7).

[d] Values are from Gallo-Rodriguez et al., J. Med. Chem., 37, 636-646 (1994).

[e] Values are from van Galen et al., Mol. Pharmacol., 45, 1101-1111 (1994). A_3 affinity measured by displacement of specific binding of [¹²⁵I]APNEA in membranes of CHO cells stably transfected with the rat A_3-cDNA. K_i values at A_1 receptors are vs. specific binding of [³H]N⁶-cyclohexyladenosine or [³H]R-PIA. K_i values at A_{2a} receptors are vs. specific binding of [³H]NECA in the presence of 50 nM CPA or vs. specific binding of [³H]CGS 21680 in rat striatal membranes.

[f] IC_{50} values (nM) vs. displacement of specific binding of [¹²⁵I]APNEA in rat brain membranes.

[g] K_d value (nM) from saturation of binding of [¹²⁵I]APNEA in membranes of CHO cells stably transfected with the rat A_3-cDNA.

109

As is apparent from the results set forth in Table
2, although the $[^{125}I]$AB-MECA is of nanomolar potency at
A_3 receptors and more potent than $[^{125}I]$APNEA, it is not
very selective for A_3 vs. A_1 or A_{2a} receptors. The
5 presence of the 4-amino group of $[^{125}I]$AB-MECA decreases
selectivity in comparison to moderately A_3 selective
agonist IB-MECA. The N^6-derivative of adenosine, APNEA,
has been used recently in pharmacological studies (Fozard
et al., supra) to stimulate A_3 receptors, although it is
10 actually A_1-selective. Until present, no mono-
substituted adenosine derivatives have been reported to
be selective for A_3 receptors. Among high affinity 5',N^6-
disubstituted adenosine derivatives, only 50 to 70-fold
selectivity for A_3 vs. A_1 receptors has been achieved.
15   . Substitution at the 2-position is often associated
with selectivity of adenosine agonists for A_{2a} vs. A_1
receptors. For example, CGS 21680 and APEC, both having
sterically bulky 2-substituents, were reported to be
highly selective for A_{2a} receptors in models of adenylate
20 cyclase (Hide et al., Mol Pharmacol., 41, 352-359
(1992)). APEC was more potent at all three adenosine
receptor subtypes than CGS 21680, and both compounds
displayed a potency order of $A_{2a} > A_3 > A_1$, consistent .
with the findings of van Galen et al., supra, that 2-
25 substitution of adenosine is well-tolerated in binding to
A_3 receptors. Among mono-substituted derivatives of
adenosine, 2-phenylamino- and 2-chloro-adenosine have K_i
values for inhibition of binding of $[^{125}I]$APNEA (N^6-[2-(4-
aminophenyl)ethyl]-adenosine) at rat A_3 receptors of 4.4
30 and 1.9 μM, respectively. Substitution at the 2-position
is also compatible with N^6-substitution for affinity at A_3
receptors. For example, 2-chloro-N^6-cyclopentyladenosine
is nearly identical in its receptor binding profile to
N^6-cyclopentyladenosine.
35   N^6-(3-Iodobenzyl)-adenosine was 2-fold selective for
A_3 vs. A_1 or A_{2a} receptors, making it is the first mono-

110

substituted adenosine analogue with any selectivity for
A_3-receptors.   2-Chloro-N^6-(3-iodobenzyl)-adenosine was 7-
fold more potent than N^6-(3-Iodobenzyl)-adenosine at A_3
receptors and of moderate selectivity.   2-Amino
5   substitution of adenosine analogues is also compatible
with N^6-substitution in A_3 receptor binding, but is not as
favorable as 2-chloro for potency and selectivity.   For
example, 2-amino-N^6-(3-iodobenzyl)adenosine was less
potent than the 2-H analogue, N^6-(3-Iodobenzyl)-
10   adenosine, by factors of: 3.2 (A_1 receptors);   6.7 (A_{2a}
receptors); and 19 (A_3 receptors).

The combination of 2-substitution with the
substituent groups of IB-MECA resulted in very high
potency and selectivity for A_3 receptors.   The A_3 affinity
15   of the 2-chloro analogue, 2-chloro-N^6-(3-iodobenzyl)-
adenosine-5'-N-methyluronamide, was 5-fold greater than
for IB-MECA.   The affinity at A_1 and A_{2a} receptors was
diminshed relative to IB-MECA by 15- and 9-fold,
respectively.   Thus, selectivities of approximately 3600-
20   fold vs. A_1 receptors and 2000-fold vs. A_{2a} receptors were
achieved.   2-Methylamino-N^6-(3-iodobenzyl)adenosine-5'-N-
methyluronamide was less potent (K_i value 3 nM), but still
highly selective for A_3 receptors.   2-Methylthio-N^6-(3-
iodobenzyl)adenosine-5'-N-methyluronamide was also highly
25   selective for A_3 receptors.

Example 58

The selectivity of several of the adenosine
derivatives vs. a nucleoside transporter previously
30   characterized in brain, namely [^{3}H]N^6-(4-
nitrobenzyl)thioinosine (NBTI), was probed.   These
experiments were carried out because of the structural
similarity of the present adenosine derivatives to
various 6-benzylethers or thioether derivatives of purine
35   ribosides, known to be high affnity antagonists of

111

adenosine uptake via this transporter.   The obtained
results are reflected in Table 3 below.

Table 3:   Inhibition of specific binding of NBTI binding at
adenosine uptake sites in rat brain membranes and the selectivity
ratio for affinity at cloned rat A_3 receptors

Compound	K_i (NBTI) [a]	Ratio of K_i (NBTI) / K_i ([125I]AB-MECA
N^6-benzyl-adenosine	203 ± 93	1.69
IB-MECA	$28,200 \pm 10,700$	22,000
2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine [Example 44]	$15,200 \pm 5,200$	66,000
N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine [Example 46]	$49,500 \pm 633$	22,000

[a] K_i values are expressed in nM as $K_i \pm$ S.E.M. for 3-4
determinations (each done in triplicate). Rat striatal membranes
were incubated for 30 min at 23 °C with 0.3 nM [^{3}H]NBTI and varying
concentrations of the nucleoside derivative in Tris buffer, pH 7.4,
in a total of 0.5 ml. Nonspecific binding was determined in the
presence of 5 μM S-(p-nitrobenzyl)-6-thioguansine.

        As is apparent from the resulting data set out in
Table 3, the simple N^6-benzyl derivative of adenosine was
found not to be selective for the receptors vs. the
adenosine transporter. In contrast, 2-chloro-N^6-(3-
iodobenzyl)-adenosine-5'-N-methyluronamide was 66,000-
fold selective for A_3 receptors vs. the Na$^+$-independent
adenosine transporter, as indicated in displacement of
[^{3}H]N^6-(4-nitrobenzyl)thioinosine binding in rat brain
membranes. Thus, in this series of 2, 6, 5'-
trisubstituted adenosine derivatives there was a high
degree of selectivity for A_3 receptors vs. potential
antagonism of adenosine uptake.
        The agonist properties of the selective ligands were
also examined. In a functional assay using membranes
from CHO cells stably transfected with rat A_3 receptors,
2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide

inhibited adenylate cyclase with an IC_{50} of 10^{-8} M. It
was a full agonist.

Example 59

5   This example describes the synthesis of 8-methoxy-
theophylline-7-β-D-ribofuranoside.

A mixture of 8-chlorotheophylline (276 mg, 1.29
mmol) and N,O-bis(trimethylsilyl)acetamide (1 ml) in dry
dichloromethane (6 ml) was stirred for 40 min at room
10   temperature under nitrogen. Solvent was removed by
rotary evaporation *in vacuo* with exclusion of moisture.
The residue was suspended in dry acetonitrile (9 ml). 1-
O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside (500 mg,
1.0 mmol), potassium nonaflate (1.0 g, 2.96 mmol), and
15   trichlorosilane (0.38 ml, 2.8 mmol) were added and the
reaction mixture was refluxed for 4 h under nitrogen.
Aqueous saturated sodium bicarbonate (30 ml) and
dichloromethane (30 ml) were added. After stirring for
15 min, two layers were separated and aqueous layer was
20   extracted with dichloromethane (3 x 30 ml). The combined
organic layer and extracts were washed with brine, dried
over anhydrous $MgSO_4$, filtered, and concentrated to
dryness. The residue was crystalized from ethyl acetate-
hexanes to yield the compound depicted as intermediate h
25   in Reaction Scheme J above (424 mg). Purification of the
filtrate on silica gel column chromatography (hexanes-
ethyl acetate, 3:1 -> 1:1) yielded additional
intermediate h (59.1 mg, 76%) as a solid: m.p. shrinks at
179-180 °C and decomposed at 258 °C. ^{1}H NMR ($CDCl_3$) d 3.38
30   (s, 3 H, N-CH_3), 3.57 (s, 3 H, N-CH_3), 4.72-4.77 (m, 2 H),
4.90 (m, 1 H), 6.17 (m, 2 H, H-5'), 6.36 (d, J = 4.6 Hz,
1 H, H-1'), 7.25-8.11 (m, 15 H, Ar).

A mixture of the compound depicted as intermediate h
in Reaction Scheme J above (212 mg, 0.33 mmol) and
35   NH_3/MeOH (20 ml) was stirred for 4 days at room
temperature. After the reaction mixture was concentrated

to dryness, the residue was purified on silica gel column
chromatography (CHCl$_3$-MeOH, 20:1) to yield 8-methoxy-
theophylline-7-β-D-ribofuranoside (33 mg, 29%) as a soft
solid. ^{1}H NMR (DMSO-d$_6$) d 3.21 (s, 3 H, NCH$_3$), 3.43 (s,
3 H, NCH$_3$), 3.45 (m, 1 H, H-5'a), 3.60 (dd, J = 11.7 and
5.2 Hz, 1 H, H-5'b), 3.79 (m, 1 H), 4.02 (m, 1 H), 4.11
(s, 4 H, OCH$_3$), 4.59 (t, J = 5.9 Hz, 1 H), 4.78 (t, J =
5.4 Hz, 1 H, exchangeable with D$_2$O, 5'-OH), 5.08 (d, J =
4.8 Hz, 1 H, exchangeable with D$_2$O, OH), 5.25 (d, J = 6.4
Hz, 1 H, exchangeable with D$_2$O, OH), 5.89 (d, J = 6.5 Hz,
1 H, H-1').

Example 60

This example describes the synthesis of 1,3-di-*n*-
butylxanthine-7-β-D-ribofuranoside.

A mixture of 1,3-di-*n*-butylxanthine (3 g, 11.35
mmol), ammonium sulfate (5 mg), and HMDS (20 ml) was
stirred in reflux for 1 h under nitrogen. HMDS was
removed by rotary evaporation *in vacuo* with exclusion of
moisture. The brown syrup was dissolved in dry
acetonitrile (70 ml). 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-
D-ribofuranoside (5.73 g, 11.36 mmol), potassium
nonaflate (15.35 g, 45.39 mmol), and trichlorosilane
(4.29 ml, 42.5 mmol) were added to the solution and the
reaction mixture was refluxed for 3 h under nitrogen.
Aqueous saturated sodium bicarbonate (30 ml) and
chloroform (30 ml) were added. After stirring for 30
min, two layers were separated and aqueous layer was
extracted with chloroform (300 ml). The combined organic
layer and extracts were washed with brine, dried over
anhydrous MgSO$_4$, filtered, and concentrated to dryness.
Purification on silica gel column chromatography
(chloroform-methanol, 250:1 -> 100:1) yielded the
compound depicted as intermediate b in Reaction Scheme J
above as pale yellow foam. ^{1}H NMR (CDCl$_3$) d 0.92-0.98 (m,
6 H, 2 x CH$_3$), 1.71-1.44 (m, 4 H, 2 x CH$_2$), 1.53-1.61 (m,

2 H, C$\underline{H}_2$), 1.68-1.76 (m, 2 H, C$\underline{H}_2$), 3.98 (t, J = 7.6 Hz, 2
H, N-C$\underline{H}_2$), 4.09 (t, J = 7.5 Hz, 2 H, N-C$\underline{H}_2$), 4.71-4.82 (m,
2 H), 4.87 (dd, J = 11.5 and 2.7 Hz, 1 H), 5.98-6.05 (m,
2 H, H-5'), 6.68 (d, J = 4.9 Hz, 1 H, H-1'), 7.34-7.62,

5   7.91-8.11 (m, 16 H, Ar and H-8).

A mixture of the intermediate b and methanolic
ammonia (saturated at 0 °C, 80 ml) was stirred for 2.5
days at room temperature. The volatiles were removed and
the residue was purified on silica gel column

10   chromatography (chloroform-methanol, 20:1) to yield 1,3-
di-n-butylxanthine-7-β-D-ribofuranoside (3.5 g, 78.1%) as
a white solid. ^{1}H NMR (DMSO-d$_6$) d 0.89 (pseudo t, J = 7.4
and 7.3 Hz, 6 H, 2 x C$\underline{H}_3$), 1.23-1.35 (m, 4 H, 2 x C$_{H2}$),
1.47-1.57 (m, 2 H, C$\underline{H}_2$), 1.59-1.69 (m, 2 H, C$\underline{H}_2$), 3.54

15   (dd, J = 12.1 and 3.7 Hz, 1 H), 3.68 (dd, J = 12.1 and
3.7 Hz, 1 H), 3.84-3.94 (m, 2 H), 3.99 (t, J = 7.2 Hz, 2
H, N-CH$_2$), 4.08 (t, J = 4.9 Hz, 1 H, N-CH$_2$), 4.33 (t, J =
4.8 Hz, 1 H), 5.05 (t, 1 H, exchangeable with D$_2$O, 5'-
OH), 5.15 (d, 1 H, exchangeable with D$_2$O, OH), 5.50 (d, 1

20   H, exchangeable with D$_2$O, OH), 6.10 (d, J = 4.8 Hz, 1 H,
H-1'), 8.45 (s, 1 H, H-8).

Example 61

This example describes the synthesis of 1-benzyl-3-

25   butylxanthine-7-β-D-ribofuranoside and 3-benzyl-1-
butylxanthine-7-β-D-ribofuranoside.

A mixture of 1-benzyl-3-butylxanthine and 3-benzyl-
1-butylxanthine (3.89 g, 13.04 mmol), ammonium sulfate (5
mg), and HMDS (20 ml) was silylated by reflux for 1 h

30   under nitrogen. HMDS was removed by rotary evaporation *in
vacuo* with exclusion of moisture. The brown syrup was
dissolved in dry acetonitrile (70 ml). 1-O-Acetyl-2,3,5-
tri-O-benzoyl-β-D-ribofuranoside (5.73 g, 11.36 mmol),
potassium nonaflate (15.35 g, 45.39 mmol), and

35   trichlorosilane (4.29 ml, 42.5 mmol) were added to the
solution and the reaction mixture was refluxed for 3.5 h

115

under nitrogen. After a workup similar to that for the
compound depicted as intermediate b in Reaction Scheme J
above in Example 60, the residue was purified on silica
gel column chromatography (chloroform-methanol, 250:1 ->
5   100:1) to yield the compounds depicted as intermediates c
[R_f = 0.76 (chloroform-methanol, 100:1)] and d in
Reaction Scheme J above [R_f = 0.70 (chloroform-methanol,
100:1)] as syrup. ^{1}H NMR (CDCl$_3$) Compound intermediate c:
d 0.91 (t, J = 7.3 Hz, 3 H, CH$_3$), 1.36 (m, 2 H, CH$_2$), 1.55
10 (m, 2 H, CH$_2$), 3.97 (t, J = 7.6 Hz, 2 H, N-CH$_2$), 4.73 (dd,
J = 11.5 and 4.3 Hz, 1 H, H-5'a), 4.79 (m, 1 H, H-4'),
4.86 (dd, J = 11.5 and 2.7 Hz, 1 H, H-5'b), 5.26 (s, 2 H,
NCH$_2$Ph), 6.01 (m, 2 H, H-2', -3'), 6.68 (d, J = 4.7 Hz, 1
H, H-1'), 7.27-7.62, 7.93-8.11 (m, 16 H, Ar and H-8).
15 Compound intermediate d: d 0.94 (t, J = 7.3 Hz, 3 H,
CH$_3$), 1.36 (m, 2 H, CH$_2$), 1.72 (m, 2 H, CH$_2$), 4.07(t, J =
7.5 Hz, 2 H, N-CH$_2$), 4.71-4.90 (m, 3 H, H-4', -5'), 5.26
(s, 2 H, N-CH$_2$Ph), 6.01 (m, 2 H, H-2', -3'), 6.71 (d, J =
4.7 Hz, 1 H, H-1'), 7.20-7.62, 7.92-8.11 (m, 16 H, Ar and
20 H-8).

A mixture of the compound depicted as intermediate c
in Reaction Scheme J above and methanolic ammonia
(saturated at 0 °C, 50 ml) was stirred for 4 days at room
temperature. The volatiles were removed and the residue
25 was purified on silica gel column chromatography
(chloroform-methanol, 20:1) to yield 1-benzyl-3-
butylxanthine-7-β-D-ribofuranoside (1.65 g, 34%) as
colorless syrup which was crystalized from
ether/methanol. ^{1}H NMR (DMSO-d$_6$) d 0.89 (pseudo t, J =
30 7.4 and 7.2 Hz, 3 H, CH$_3$), 1.31 (m, 2 H, CH$_2$), 1.53 (m, 2
H, CH$_2$), 3.55 (dt, J = 12.5 and 4.7 Hz, 1 H, H-5'a), 3.69
(dt, J = 12.0 and 4.7 Hz, 1 H, H-5'b), 3.89 (m, 1 H, H-
4'), 3.91 (t, J = 7.2 Hz, 2 H, N-CH$_2$), 4.09 (q, J = 4.9
Hz, 1 H, H-3'), 4.34 (q, J = 5.0 Hz, 1 H, H-2'), 5.04 (t,
35 J = 5.3 Hz, 1 H, exchangeable with D$_2$O, 5'-OH), 5.14 (d,
J = 5.3 Hz, 1 H, exchangeable with D$_2$O, OH), 5.18 (s, 2

116

H, N-CH₂Ph), 5.48 (d, *J* = 5.8 Hz, 1 H, exchangeable with
D₂O, OH), 6.12 (d, *J* = 4.6 Hz, 1 H, H-1'), 7.24-7.36 (m,
5 H, Bn), 8.48 (s, 1 H, H-8).

     A mixture of intermediates c and d in methanolic
ammonia (saturated at 0 °C, 30 ml) was stirred for 3 days
at room temperature. The volatiles were removed and the
residue was purified on silica gel column chromatography
(chloroform-methanol, 24:1) to yield a mixture of 1-
benzyl-3-butylxanthine-7-β-D-ribofuranoside and 3-benzyl-
1-butylxanthine-7-β-D-ribofuranoside (0.75 g, 15%) which
was crystalized from ether/methanol. Part of the solid
was dissolved in ethyl acetate with heating and sit on
bench for 5 h. The formed white cotten like solid was
filtered and dried *in vacuo* to yield 3-benzyl-1-
butylxanthine-7-β-D-ribofuranoside. ¹H NMR (DMSO-d₆) d
0.89 (pseudo t, *J* = 7.4 and 7.2 Hz, 3 H, CH₃), 1.30 (m, 2
H, CH₂), 1.63 (m, 2 H, CH₂), 3.55 (dt, *J* = 11.8 and 4.6
Hz, 1 H, H-5'a), 3.68 (dt, *J* = 12.0 and 4.0 Hz, 1 H, H-
5'b), 3.91 (q, *J* = 4.0 Hz, 1 H, H-4'), 4.00 (t, *J* = 7.3
Hz, 1 H, N-CH₂), 4.09 (q, *J* = 4.9 Hz, 1 H, H-3'), 4.34
(q, *J* = 5.0 Hz, 1 H, H-2'), 5.04 (t, *J* = 5.3 Hz, 1 H,
exchangeable with D₂O, 5'-OH), 5.07 (s, 2 H, N-CH₂Ph),
5.14 (d, *J* = 5.3 Hz, 1 H, exchangeable with D₂O, OH),
5.48 (d, *J* = 5.8 Hz, 1 H, exchangeable with D₂O, OH),
6.12 (d, *J* = 4.6 Hz, 1 H, H-1'), 7.23-7.30 (m, 5 H, Bn),
8.50 (s, 1 H, H-8).

Example 62

     This example describes the synthesis of 1,3-di-*n*-
butyl-2-thio-xanthine-7-β-D-ribofuranoside.

     A solution of 1,3-di-*n*-butyl-2-thio-xanthine (0.325
g, 1.16 mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.96
ml, 2.8 mmol) in dry methylene chloride (6 ml) was
stirred for 30 min at room temperature under nitrogen.
After the reaction mixture was concentrated to dryness in
vacuo, the residue was dissolved in dry acetonitrile (10

ml). 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside
(0.5 g, 0.99 mmol), potassium nonaflate (1 g, 2.96 mmol),
and trichlorosilane (0.38 ml, 2.8 mmol) were added to the
solution and the reaction mixture was refluxed for 18 h

5   under nitrogen. After a workup similar to that for the
compound of Example 60, the residue was purified on
silica gel column chromatography (hexanes-ethyl acetate,
3:1) to yield the compound depicted as intermediate e in
Reaction Scheme J above (0.58 g, 82.4%) as colorless

10   foam. ^{1}H NMR (CDCl$_3$) d 0.91-1.00 (m, 6 H, 2 x CH$_3$), 1.41
(sixtett, 4 H, 2 x CH$_2$), 1.63-1.76 (m, 2 H, CH$_2$), 1.79-
1.84 (m, 2 H, CH$_2$), 4.54 (pseudo t, J = 7.9 and 7.6 Hz, 2
H, N-CH$_2$), 4.64 (pseudo t, J = 8.2 and 7.6 Hz, 2 H, N-
CH$_2$), 4.74 (dd, J = 11.4 and 3.7 Hz, 1 H), 4.81 (m, 1 H),

15   4.88 (dd, J = 11.4 and 2,5 Hz, 1 H), 5.99 (m, 2 H), 6.70
(d, J = 4.4 Hz, 1 H, H-1'), 7.34-7.63, 7.94-8.11 (m, 16
H, Ar and H-8).

A mixture of intermediate e (555 mg, 0.78 mmol) and
methanolic ammonia (saturated at 0 °C, 45 ml) was stirred

20   for 22 h at room temperature. The volatiles were removed
and the residue was purified on silica gel column
chromatography (chloroform-methanol, 20:1) to yield 1,3-
di-n-butyl-2-thio-xanthine-7-β-D-ribofuranoside (276 mg,
85.3%) as a white solid. ^{1}H NMR (DMSO-d$_6$) d 0.93 (t, J =

25   7.3 Hz, 6 H, 2 x CH$_3$), 1.31-1.42 (m, 4 H, 2 x CH$_2$), 1.60-
1.69 (m, 2 H, CH$_2$), 1.72-1.79 (m, 2 H, CH$_2$), 3.57 (dd, J =
12.1 and 3.5 Hz, 1 H, H-5'a), 3.71 (dd, J = 12.1 and 3.5
Hz, 1 H, H-5'b), 3.94 (m, 1 H, H-4'), 4.10 (t, J = 4.9
Hz, 1 H, H-3'), 4.33 (t, J = 4.6 Hz, 1 H, H-2'), 4.47

30   (pseudo t, J = 8.3 and 7.2 Hz, 2 H, N-CH$_2$), 4.57 (pseudo
t, J = 7.8and 7.5 Hz, 2 H, N-CH$_2$), 5.09 (br s, 1 H,
exchangeable with D$_2$O, OH), 5.15 (br s, 1 H, exchangeable
with D$_2$O, OH), 5.52 (br s, 1 H, exchangeable with D$_2$O,
OH), 6.14 (d, J = 4.0 Hz, 1 H, H-1'), 8.63 (s, 1 H, H-

35   8).

118

Example 63

This example describes the synthesis of 1,3-di-*n*-pentylxanthine-7-β-D-ribofuranoside.

A solution of 1,3-di-*n*-pentylxanthine (0.377 g, 1.29
5   mmol) and *N,O*-bis(trimethylsilyl)acetamide (0.96 ml, 2.8
mmol) in dry methylene chloride (5 ml) was stirred for 30
min at room temperature under nitrogen. After the
reaction mixture was concentrated to dryness in vacuo,
the residue was dissolved in dry acetonitrile (10 ml).
10   1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside (0.5 g,
0.99 mmol), potassium nonaflate (1 g, 2.96 mmol), and
trichlorosilane (0.38 ml, 2.8 mmol) were added to the
solution and the reaction mixture was refluxed for 20 h
under nitrogen. After a workup similar to that for the
15   compound of Example 60, the residue was purified on
silica gel column chromatography (hexanes-ethyl acetate,
3:1) to yield the compound depicted as intermediate f in
Reaction Scheme J above (0.599 g, 82.1%) as colorless
foam. ^{1}H NMR (CDCl$_3$) d 0.87 (m, 6 H, 2 x CH$_3$), 1.25-1.35
20   (m, 8 H, 4 x CH$_2$), 1.57-1.60 (m, 2 H, CH$_2$), 1.72-1.77 (m,
2 H, CH$_2$), 3.96 (pseudo t, J = 7.9 and 7.5 Hz, 2 H, N-
CH$_2$), 4.08 (pseudo t, J = 8.2 and 7.1 Hz, 2 H, N-CH$_2$),
4.72 (dd, J = 11.8 and 4.6 Hz, 1 H), 4.79 (m, 1 H), 4.86
(dd, J = 11.4 and 2,5 Hz, 1 H), 6.02 (m, 2 H), 6.69 (d, J
25   = 4.7 Hz, 1 H, H-1'), 7.34-7.62, 7.91-8.11 (m, 16 H, Ar
and H-8).

A mixture of intermediate f (570 mg, 0.77 mmol) and
methanolic ammonia (saturated at 0 °C, 15 ml) was stirred
for 67 h at room temperature. The volatiles were removed
30   and the residue was purified on silica gel column
chromatography (chloroform-methanol, 20:1) to yield 1,3-
di-*n*-pentylxanthine-7-β-D-ribofuranoside (280 mg, 85.4%)
as a white solid. ^{1}H NMR (DMSO-d$_6$) d 0.83-0.88 (m, 6 H, 2
x CH$_3$), 1.23-1.32 (m, 8 H, 4 x CH$_2$), 1.49-1.59 (m, 2 H,
35   CH$_2$), 1.61-1.71 (m, 2 H, CH$_2$), 3.51-3.56 (m, 1 H, H-5'a),
3.66-3.71 (m, 1 H, H-5'b), 3.86 (pseudo t, J = 7.5 and

7.2, 1 H, N-C$\underline{H}_2$), 3.91 (m, 1 H, H-4'), 3.98 (t, J = 7.2
Hz, 1 H, N-CH$_2$), 4.09 (q J = 4.9 Hz, 1 H, H-3'), 4.34 (q,
J = 5.1 Hz, 1 H, H-2'), 5.04 (pseudo t, J = 5.2 and 5.0
Hz, 1 H, exchangeable with D$_2$O, 5'-OH), 5.14 (d, J = 5.4
Hz, 1 H, exchangeable with D$_2$O, OH), 5.46 (d, J = 6.1 Hz,
1 H, exchangeable with D$_2$O, OH), 6.11 (d, J = 4.7 Hz, 1
H, H-1'), 8.46 (s, 1 H, H-8).

Example 64

This example describes the synthesis of 1,3-di-n-
hexylxanthine-7-β-D-ribofuranoside.

A solution of 1,3-di-n-hexylxanthine (0.428 g, 1.34
mmol) and N,O-bis(trimethylsilyl)acetamide (0.99 ml, 2.97
mmol) in dry methylene chloride (5 ml) was stirred for 40
min at room temperature under nitrogen. After the
reaction mixture was concentrated to dryness *in vacuo*,
the residue was dissolved in dry acetonitrile (10 ml).
1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside (0.5 g,
0.99 mmol), potassium nonaflate (1 g, 2.96 mmol), and
trichlorosilane (0.38 ml, 2.8 mmol) were added to the
solution and the reaction mixture was refluxed for 14 h
under nitrogen. After a workup similar to that for the
compound of Example 60, the residue was purified on
silica gel column chromatography (hexanes-ethyl acetate,
3:1) to yield the compound depicted as intermediate g in
Reaction Scheme J above (0.526 g, 69.4%) as colorless
foam. ^{1}H NMR (CDCl$_3$) d 0.77-0.87 (m, 6 H, 2 x CH$_3$), 1.22-
1.40 (m, 12 H, 6 x CH$_2$), 1.54-1.59 (m, 2 H, C$\underline{H}_2$), 1.69-
1.74 (m, 2 H, C$\underline{H}_2$), 3.96 (t, J = 7.6 Hz, 2 H, N-C$\underline{H}_2$), 4.08
(t, J = 7.6 Hz, 2 H, N-C$\underline{H}_2$), 4.74 (dd, J = 11.6 and 4.3
Hz, 1 H, H-5'a), 4.80 (m, 1 H, H-4'), 4.86 (dd, J = 11.6
and 2,5 Hz, 1 H, H-5'b), 6.02 (m, 2 H), 6.68 (d, J = 4.8
Hz, 1 H, H-1'), 7.34-7.62, 7.91-8.11 (m, 16 H, Ar and H-
8).

A mixture of intermediate g (520 mg, 0.68 mmol) and
methanolic ammonia (saturated at 0 °C, 15 ml) was stirred

for 4 days at room temperature. The volatiles were
removed and the residue was purified on silica gel column
chromatography (chloroform-methanol, 20:1) to yield 1,3-
di-*n*-hexylxanthine-7-β-D-ribofuranoside (240 mg, 80%) as

5   a white solid. ^{1}H NMR (DMSO-d$_6$) d 0.85 (m, 6 H, 2 x CH$_3$),
1.27 (m, 12 H, 6 x CH$_2$), 1.53 (m, 2 H, CH$_2$), 1.64 (m, 2 H,
CH$_2$), 3.56 (dt, 1J = 11.8 and 4.7 Hz, 1 H, H-5'a), 3.68
(dt, J = 11.7 and 4.4 Hz, 1 H, H-5'b), 3.86 (pseudo t, J
= 7.8 and 6.9, 1 H, N-CH$_2$), 3.91 (m, 1 H, H-4'), 3.98

10   (pseudo t, J = 7.5 and 7.1 Hz, 1 H, N-CH$_2$), 4.08 (q J =
5.0 Hz, 1 H, H-3'), 4.34 (q, J = 5.1 Hz, 1 H, H-2'), 5.05
(pseudo t, J = 5.4 and 5.3 Hz, 1 H, exchangeable with
D$_2$O, 5'-OH), 5.15 (d, J = 5.5 Hz, 1 H, exchangeable with
D$_2$O, OH), 5.48 (d, J = 5.8 Hz, 1 H, exchangeable with D$_2$O,

15   OH), 6.11 (d, J = 4.7 Hz, 1 H, H-1'), 8.47 (s, 1 H, H-
8).

Example 65

     This example describes the synthesis of methyl 1,3-
20   di-*n*-butylxanthine-7-β-D-ribofuronate.

     A solution of methyl 1,3-di-*n*-butylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (0 mg, 0.065 mmol) in
88% formic acid (3 ml) was stirred for 3 h at room
temperature and the solvent was removed by rotary

25   evaporation. The residue was purified by preparative TLC
(chloroform-methanol, 10:1) to yield methyl 1,3-di-*n*-
butylxanthine-7-β-D-ribofuronate (14.8 mg, 57%) as a
colorless solid. ^{1}H NMR (DMSO-d$_6$) d 0.90 and 0.91 (2 x t,
J = 7.5 Hz, 2 x 3 H, 2 x CH$_3$), 1.23-1.35 (m, 4 H, 2 x

30   CH$_2$), 1.47-1.57 (m, 2 H, CH$_2$), 1.60-1.70 (m, 2 H, CH$_2$),
3.72 (s, 3 H, -OCH$_3$), 3.87 (t, J = 7.3 Hz, 2 H, N-CH$_2$),
4.00 (t, J = 7.2 Hz, 2 H, N-CH$_2$), 4.29 (m, 1 H), 4.49 (m,
2 H), 5.73 (d, J = 6.1 Hz, exchangeable with D$_2$O, 1 H,
OH), 5.81 (d, J = 4.7 Hz, exchangeable with D$_2$O, 1 H,

35   OH), 6.29(d, J = 5.4 Hz, 1 H, H-1'), 8.46 (s, 1 H, H-8).

121

Example 66

This example describes the synthesis of 1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide.

A mixture of methyl 1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronate (87 mg, 0.19 mmol) and methanolic ammonia (10 ml, saturated at 0 °C) was stirred at 85 °C for 18 h in a sealed bottle. After cooling, the volatiles were removed by rotary evaporation and the residue was purified by preparative TLC (chloroform-methanol, 20:1) to yield 1,3-di-*n*-butylxanthine-7-β-D-2,3-isopropylidene-ribofuronamide (67.5 mg, 80.2%) as a syrup. ^{1}H NMR (CDCl$_3$) d 0.93-0.99 (m, 6 H, 2 x C$\underline{H}_3$), 1.32-1.47 (m, 7 H, 2 x C$\underline{H}_2$ and isopropylidene), 1.55-1.66 (m, 5 H, C$\underline{H}_2$ and isopropylidene), 1.69-1.79 (m, 2 H, C$\underline{H}_2$), 4.00 (pseudo t, J = 7.8 and 7.3 Hz, 2 H, N-C$\underline{H}_2$), 4.12 (pseudo t, J = 7.5 and 7.3 Hz, 2 H, N-C$\underline{H}_2$), 4.59 (d, J = 3.6 Hz, 1 H), 5.15 (dd, J = 7.1 and 3.9 Hz, 1 H), 5.28 (dd, J = 7.1 and 3.6 Hz, 1 H), 5.35 and 6.86 (2 x br s, 2 x 1 H, NH$_2$'), 5.93 (d, J = 3.9 Hz, 1 H, H-1'), 7.74 (s, 1 H, H-8). Anal. Calcd for C$_{21}$H$_{31}$N$_5$O$_6$-0.2(C$_2$H$_5$)$_2$O: C, 56.39; H, 7.16; N, 15.08; Found: C, 56.63; H, 7.22; N, 15.13.

A deisopropylidenation procedure similar to that for the compound of Example 65 with 56 mg of protected compound and followed by crystallization with ether-methanol yielded 1,3-di-n-butylxanthine-7-β-D-ribofuronamide (20 mg, 40%) as a slightly yellow solid. ^{1}H NMR (DMSO-d$_6$) d 0.88-0.92 (m, 6 H, 2 x C$\underline{H}_3$), 1.23-1.37 (m, 4 H, 2 x C$\underline{H}_2$), 1.47-1.57 (m, 2 H, C$\underline{H}_2$), 1.60-1.70 (m, 2 H, C$\underline{H}_2$), 3.87 (pseudo t, J = 7.5 and 7.2 Hz, 2 H, N-C$\underline{H}_2$), 4.00 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, N-C$\underline{H}_2$), 4.14-4.15 (m, 1 H, H-3'), 4.29 (d, J = 3.7 Hz, 1 H, H-4'), 4.40-4.43 (m, 1 H, H-2'), 5.58 (d, J = 5.0 Hz, exchangeable with D$_2$O, 1 H, OH), 5.62 (d, J = 6.1 Hz, exchangeable with D$_2$O, 1 H, OH), 6.19 (d, J = 5.8 Hz, 1 H, H-1'), 7.43 and 7.62 (2 x br s, exchangeable with D$_2$O, 2 x 1 H, NH$_2$), 8.67 (s, 1 H, H-8).

122

Example 67

This example describes the synthesis of N-methyl
1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide.

To a solution of methyl 1,3-di-*n*-butylxanthine-7-β-
5   D-2,3-O-isopropylidene-ribofuronate (50 mg, 0.11 mmol) in
methanol (10 ml) was bubbled methylamine for 5 min at -78
°C (1/3 increase in volume). The reaction mixture was
heated for 17 h at 85 °C in a sealed tube. After
evaporation of the solvent, the slightly yellow residue
10   was purified by preparative TLC (chloroform-methanol,
20:1) to yield N-methyl 1,3-di-*n*-butylxanthine-7-β-D-2,3-
isopropylidene-ribofuronamide (23.1 mg, 46.3%) as a foam.
^{1}H NMR (CDCl$_3$) d 0.96 and 0.97 (2 x t, J = 7.3 Hz, 2 x 3
H, 2 x C$\underline{H}_3$), 1.34-1.44 (m, 7 H, 2 x C$\underline{H}_2$ and
15   isopropylidene), 1.51-1.69 (m, 5 H, C$\underline{H}_2$ and
isopropylidene), 1.72-1.79 (m, 2 H, C$\underline{H}_2$), 2.79 (d, J =
4.9 Hz, 3 H, -NH-C$\underline{H}_3$), 4.01 (t, J = 7.5 Hz, 2 H, N-CH$_2$),
4.12 (pseudo t, J = 7.6 and 7.3 Hz, 2 H, N-C$\underline{H}_2$), 4.60 (d,
J = 3.0 Hz, 1 H), 5.14 (dd, J = 6.9 and 4.0 Hz, 1 H),
20   5.21 (dd, J = 6.9 and 3.1 Hz, 1 H), 5.91 (d, J = 4.0 Hz,
1 H, H-1'), 6.92 (m, 1 H, NH), 7.72 (s, 1 H, H-8).

A mixture of the isopropylidene compound (20 mg,
0.043 mmol) and 88% formic acid (3 ml) reacted for 6 h at
room temperature. After the reaction mixture was
25   concentrated to dryness, the residue was coevaporated
with toluene (2 x 5 ml) and triturated with ether to
yield N-methyl 1,3-di-*n*-butylxanthine-7-β-D-
ribofuronamide (12.7 mg, 69.5%) as a colorless solid. ^{1}H
NMR (DMSO-d$_6$) d 0.88-0.92 (m, 6 H, 2 x C$\underline{H}_3$), 1.26-1.35 (m,
30   4 H, 2 x C$\underline{H}_2$), 1.48-1.58 (m, 2 H, C$\underline{H}_2$), 1.61-1.70 (m, 2 H,
C$\underline{H}_2$), 2.64 (d, J = 4.3 Hz, 3 H, -NHC$\underline{H}_3$), 3.88 (t, J = 7.4
Hz, 2 H, N-C$\underline{H}_2$), 4.01 (pseudo t, J = 7.3 and 7.1 Hz, 2 H,
N-C$\underline{H}_2$), 4.16-4.19 (m, 1 H), 4.30 (d, J = 3.6 Hz, 1 H),
4.44-4.47 (m, 1 H), 5.60 (2 x d, J = 6.5 and 5.5 Hz,
35   exchangeable with D$_2$O, 2 x 1 H, 2 x OH), 6.19 (d, J =

123

5.4 Hz, 1 H, H-1'), 8.11 (q, J = 4.3 Hz, exchangeable
with D_2O, 1 H, NH), 8.65 (s, 1 H, H-8).

<u>Example 68</u>

5   This example describes the synthesis of N-methyl
1,3-di-n-pentylxanthine-7-β-D-ribofuronamide.

       A solution of methyl-1,3-di-n-pentylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (65.4 mg, 0.13 mmol)
and 2 M methylamine/THF (2 ml) was heated for 18 h at 85
10   °C in a sealed tube. After evaporation of the solvent,
the slightly yellow residue was purified by preparative
TLC (chloroform-methanol, 20:1) to yield N-methyl 1,3-di-
n-pentylxanthine-7-β-D-2,3-isopropylidene-ribofuronamide
(52 mg, 80%) as a foam. ^{1}H NMR ($CDCl_3$) d 0.91 (t, J = 6.5
15   Hz, 6 H, 2 x C$\underline{H}_3$), 1.25-1.36 (m, 11 H, 4 x C$\underline{H}_2$ and
isopropylidene), 1.56-1.61 (m, 5 H, C$\underline{H}_2$ and
isopropylidene), 1.71-1.78 (m, 2 H, C$\underline{H}_2$), 2.79 (d, J =
5.0 Hz, 3 H, -NH-C$\underline{H}_3$), 4.00 (t, J = 7.7 Hz, 2 H, N-CH_2),
4.11 (pseudo t, J = 8.0 and 7.2 Hz, 2 H, N-C$\underline{H}_2$), 4.61 (d,
20   J = 3.1 Hz, 1 H), 5.14 (dd, J = 6.7 and 4.0 Hz, 1 H),
5.21 (dd, J = 6.8 and 3.2 Hz, 1 H), 5.91 (d, J = 3.7 Hz,
1 H, H-1'), 6.93 (br d, J = 4.0 Hz, 1 H, NH), 7.73 (s, 1
H, H-8).

       A mixture of the isopropylidene compound (46 mg,
25   0.094 mmol) and 88% formic acid (5 ml) reacted for 2.5 h
at room temperature. After the reaction mixture was
concentrated to dryness, the residue was purified on
preparative TLC (chloroform-methanol, 10:1) to yield N-
methyl 1,3-di-n-pentylxanthine-7-β-D-ribofuronamide (32.6
30   mg, 78%) as a colorless solid. ^{1}H NMR (DMSO-d_6) d 0.86
(pseudo t, J = 7.1 and 6.2 Hz, 6 H, 2 x C$\underline{H}_3$), 1.24-1.29
(m, 8 H, 4 x C$\underline{H}_2$), 1.50-1.63 (m, 2 H, C$\underline{H}_2$), 1.65-1.72 (m,
2 H, C$\underline{H}_2$), 2.64 (d, J = 4.7 Hz, 3 H, -NHC$\underline{H}_3$), 3.87 (t, J =
7.3 Hz, 2 H, N-C$\underline{H}_2$), 4.00 (pseudo t, J = 7.3 and 7.2 Hz,
35   2 H, N-C$\underline{H}_2$), 4.16-4.18 (m, 1 H), 4.31 (d, J = 3.6 Hz, 1
H), 4.38-4.54 (m, 1 H), 5.60 (2 x d, J = 5.8 and 5.1 Hz,

124

exchangeable with D$_2$O, 2 x 1 H, 2 x OH), 6.20 (d, J = 5.7
Hz, 1 H, H-1'), 8.11 (q, J = 4.5 Hz, exchangeable with
D$_2$O, 1 H, NH), 8.65 (s, 1 H, H-8).

5   Example 69
        This example describes the synthesis of N-methyl
1,3-di-n-hexylxanthine-7-β-D-ribofuronamide.
        A mixture of methyl 1,3-di-n-hexylxanthine-7-β-D-
2,3-O-isopropylidene-ribofuronate (62 mg, 0.12 mmol) and
10   CH$_3$NH$_2$/THF (2 ml) was heated for 16 h at 85 °C in a sealed
tube. After evaporation of the volatiles, the slightly
yellow residue was purified by preparative TLC
(chloroform-methanol, 20:1) to yield N-methyl 1,3-di-n-
hexylxanthine-7-β-D-2,3-isopropylidene-ribofuronamide
15   (45.4 mg, 73%) as thick oil. ^{1}H NMR (CDCl$_3$) d 0.86-0.91
(pseudo t, J = 6.8 and 6.2 Hz, 6 H, 2 x CH$_3$), 1.25-1.36
(m, 15 H, 6 x CH$_2$ and isopropylidene), 1.60 (m, 5 H, CH$_2$
and isopropylidene), 1.70-1.77 (m, 2 H, CH$_2$), 2.79 (d, J
= 4.8 Hz, 3 H, -NH-CH$_3$), 3.99 (t, J = 7.8 Hz, 2 H, N-CH$_2$),
20   4.11 (pseudo t, J = 7.9 and 7.2 Hz, 2 H, N-CH$_2$), 4.60 (d,
J = 3.5 Hz, 1 H, H-4'), 5.14 (dd, J = 7.1 and 3.9 Hz, 1
H, H-3'), 5.20 (dd, J = 6.9 and 3.1 Hz, 1 H, H-2'), 5.91
(d, J = 3.7 Hz, 1 H, H-1'), 6.92 (br d, J = 4.3 Hz, 1 H,
NH), 7.72 (s, 1 H, H-8).
25   A mixture of the isopropylidene compound (42.5 mg,
0.082 mmol) and 88% formic acid (2 ml) reacted for 6 h at
room temperature. After the reaction mixture was
concentrated to dryness, the residue was evaporated to
dryness and dried in $vacuo$ to yield N-methyl 1,3-di-n-
30   hexylxanthine-7-β-D-ribofuronamide (35 mg, 89%) as a
colorless solid. ^{1}H NMR (DMSO-d$_6$) d 0.84 (m, 6 H, 2 x
CH$_3$), 1.28 (m, 12 H, 6 x CH$_2$), 1.54 (m, 2 H, CH$_2$), 1.65
(m, 2 H, CH$_2$), 2.64 (d, J = 4.4 Hz, 3 H, -NHCH$_3$), 3.87
(pseudo t, J = 7.8 and 6.6 Hz, 2 H, N-CH$_2$), 4.00 (pseudo
35   t, J = 7.2 and 6.9 Hz, 2 H, N-CH$_2$), 4.18 (m, 1 H, H-3'),
4.30 (d, J = 3.5 Hz, 1 H, H-4'), 4.46 (m, 1 H, H-2'),

125

5.60 (m, exchangeable with D_2O, 2 H, 2 x OH), 6.19 (d, J = 5.5 Hz, 1 H, H-1'), 8.11 (br d, J = 4.4 Hz, exchangeable with D_2O, 1 H, NH), 8.65 (s, 1 H, H-8).

5

Example 70

This example describes the synthesis of N,N-dimethyl 1,3-di-n-butylxanthine-7-β-D-ribofuronamide.

A solution of methyl 1,3-di-n-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronate (77 mg, 0.166 mmol) and

10   25% dimethylamine/methanol (10 ml, dissolved at -78 °C) was heated for 15 h at 75 °C in a sealed tube. After evaporation of the volatiles, the residue was purified by preparative TLC (chloroform-methanol, 20:1) to yield N,N-dimethyl 1,3-di-n-butylxanthine-7-β-D-2,3-isopropylidene-

15   ribofuronamide (50 mg, 63.2%) as a thick syrup. ^{1}H NMR ($CDCl_3$) d 0.92-0.98 (m, 6 H, 2 x CH_3), 1.33-1.46 (m, 7 H, 2 x CH_2 and isopropylidene), 1.58-1.68 (m, 5 H, CH_2 and isopropylidene), 1.71-1.78 (m, 2 H, CH_2), 2.93 and 3.11 [2 x s, 2 x 3 H, -N(CH_3)$_2$], 4.01 (t, J = 7.8 Hz, 2 H, N-

20   CH_2), 4.09 (t, J = 7.6 Hz, 2 H, N-CH_2), 5.07 (s, 1 H), 5.15 (d, J = 6.3 Hz, 1 H), 5.33 (m, 1 H), 6.69 (s, 1 H, H-1'), 8.01 (s, 1 H, H-8).

A deisopropylidenation similar to that for the compound of Example 65 with 40 mg of protected compound

25   and purification on preparative TLC (chloroform-methanol, 10:1) yielded N,N-dimethyl 1,3-di-n-butylxanthine-7-β-D-ribofuronamide (30 mg, 82%) as a colorless solid. ^{1}H NMR (DMSO-d_6) d 0.88-0.92 (m, 6 H, 2 x CH_3), 1.24-1.37 (m, 4 H, 2 x CH_2), 1.48-1.57 (m, 2 H, CH_2), 1.60-1.70 (m, 2 H,

30   CH_2), 2.89 and 3.05 [2 x s, 2 x 3 H, N(CH_3)$_2$], 3.87 (pseudo t, J = 7.5 and 7.1 Hz, 2 H, N-CH_2), 4.00 (pseudo t, J = 7.2 and 6.9 Hz, 2 H, N-CH_2), 4.22 (dd, J = 8.7 and 4.2 Hz, 1 H), 4.29-4.34 (m, 1 H), 4.90 (d, J = 3.9 Hz, 1 H), 5.65 and 5.69 (2 x d, J = 5.5 and 6.0 Hz,

35   exchangeable with D_2O, 2 H, 2 x OH), 6.32 (d, J = 4.7 Hz, 1 H, H-1'), 8.75 (s, 1 H, H-8).

126

Example 71

This example describes the synthesis of N-ethyl 1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide.

A mixture of methyl 1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronate (70 mg, 0.15 mmol) and 25% ethylamine/methanol (10 ml, dissolved at -78 °C) was heated for 18 h at 85 °C in a sealed tube. After evaporation of the solvent, the slightly yellow residue was purified by preparative TLC (chloroform-methanol, 20:1) to yield N-ethyl 1,3-di-*n*-butylxanthine-7-β-D-2,3-isopropylidene-ribofuronamide (55.7 mg, 77.4%) as a syrup. ^{1}H NMR (CDCl$_3$) d 0.93-0.99 (m, 6 H, 2 x C$\underline{H}_3$), 1.11 (t, J = 7.3 Hz, 3 H, -NHCH$_2$C$\underline{H}_3$), 1.33-1.41 (m, 7 H, 2 x C$\underline{H}_2$ and isopropylidene), 1.58-1.70 (m, 5 H, C$\underline{H}_2$ and isopropylidene), 1.72-1.77 (m, 2 H, C$\underline{H}_2$), 3.24-3.32 (m, 2 H, -NHC$\underline{H}_2$CH$_3$), 4.00 (pseudo t, J = 7.9 and 7.8 Hz, 2 H, N-C$\underline{H}_2$), 4.12 (pseudo t, J = 7.5 and 7.3 Hz, 2 H, N-C$\underline{H}_2$), 4.58 (d, J = 3.5 Hz, 1 H), 5.13 (dd, J = 6.7 and 3.8 Hz, 1 H), 5.18 (dd, J = 6.7 and 3.1 Hz, 1 H), 5.91 (d, J = 4.2 Hz, 1 H, H-1'), 6.96 (m, 1 H, NH), 7.73 (s, 1 H, H-8). Anal. Calcd for C$_{23}$H$_{35}$N$_5$O$_6$-0.5(C$_2$H$_5$)$_2$O: C, 58.35; H, 7.84; N, 13.61; Found: C, 58.06; H, 7.57; N, 13.54.

A deisopropylidenation similar to that for the compound of Example 65 with 55 mg of protected compound and crystalization with ether-methanol yielded N-ethyl 1,3-di-*n*-butylxanthine-7-β-D-ribofuronamide (25.1 mg, 73.8%) as a colorless solid. ^{1}H NMR (DMSO-d$_6$) d 0.88-0.93 (m, 6 H, 2 x C$\underline{H}_3$), 1.05 (t, J = 7.4 Hz, 3 H, NHCH$_2$C$\underline{H}_3$), 1.24-1.35 (m, 4 H, 2 x C$\underline{H}_2$), 1.48-1.58 (m, 2 H, C$\underline{H}_2$), 1.61-1.71 (m, 2 H, C$\underline{H}_2$), 3.09-3.19 (m, 2 H, NHC$\underline{H}_2$CH$_3$), 3.88 (pseudo t, J = 7.5 and 7.2 Hz, 2 H, N-C$\underline{H}_2$), 4.01 (t, J = 7.2 Hz, 2 H, N-C$\underline{H}_2$), 4.14-4.19 (m, 1 H), 4.29 (d, J = 3.5 Hz, 1 H), 4.43-4.48 (m, 1 H), 5.57 and 5.59 (2 x d, J = 6.4 and 5.5 Hz, exchangeable with D$_2$O, 2 H, 2 x OH), 6.17 (d, J = 5.5 Hz, 1 H, H-1'), 8.18 (t, J = 5.4 Hz, exchangeable with D$_2$O, 1 H, NH), 8.64 (s, 1 H, H-8).

127

Example 72

This example describes the synthesis of 1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside.

A mixture of 1,3-di-*n*-butylxanthine-7-β-D-ribofuranoside (1.56 g, 3.94 mmol), *p*-toluenesulfonic acid (1.3 g, 1.58 mmol), and dry acetone (25 ml) was stirred for 7 h at room temperature and kept in refrigerator for 2 days. After stirring for 1 h at room temperature, the reaction mixture was neutralized by triethylamine and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (chloroform-methanol, 100:0 -> 50:1) to yield 1,3-di-*n*-butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside (1.7 g, 98.9%) as a thick syrup. ^{1}H NMR (DMSO-d_6) d 0.89 (t, J = 7.3 Hz, 6 H, 2 x CH$_3$), 1.21-1.35 (m, 7 H, 2 x CH$_2$ and isopropylidene), 1.47-1.56 (m, 5 H, CH$_2$ and isopropylidene), 1.59-1.69 (m, 2 H, CH$_2$), 3.49-3.57 (m, 2 H, H-5'), 3.86 (pseudo t, J = 7.6 and 7.2 Hz, 2 H, N-CH$_2$), 3.99 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, N-CH$_2$), 4.17 (dd, J = 8.1 and 4.9 Hz, 1 H), 4.88 (dd, J = 6.4 and 3.1 Hz, 1 H), 5.07 (t, J = 5.2 Hz, exchangeable with D$_2$O, 1 H, 5'-OH), 5.15 (dd, J = 6.4 and 3.5 Hz, 1 H), 6.29 (d, J = 3.0 Hz, 1 H, H-1'), 8.40 (s, 1 H, H-8).

Example 73

This example describes the synthesis of 1,3-di-*n*-pentylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside.

A solution of 1,3-di-*n*-pentylxanthine-7-β-D-ribofuranoside (145.2 mg, 0.34 mmol), (1R)-(-)-camphorsulphonic acid (80 mg, 0.34 mmol) in dry acetone (5 ml) was stirred for 21 h at room temperature. The solvent was removed by rotary evaporation and the residue was purified by silica gel column chromatography (chloroform-methanol, 20:1) to yield 1,3-di-*n*-pentylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside

128

(147.2 mg, 92.5%) as a thick syrup. ^{1}H NMR (CDCl$_3$) d 0.90
(pseudo t, J = 7.1 and 6.4 Hz, 6 H, 2 x CH$_3$), 1.34-1.38
(m, 11 H, 4 x CH$_2$ and isopropylidene), 1.59-1.69 (m, 5 H,
CH$_2$ and isopropylidene), 1.70-1.78 (m, 2 H, CH$_2$), 3.79-
5   4.03 (m, 5 H, H-4', 5' and N-CH$_2$), 4.11 (pseudo t, J =
7.6 and 7.3 Hz, 2 H, N-CH$_2$), 4.34 (br s, 1 H), 5.12 (dd,
J = 7.2 and 4.7 Hz, 1 H, H-2'), 5.21 (dd, J = 10.1 and
3.0 Hz, 1 H, H-3'), 5.75 (d, J = 5.0 Hz, 1 H, H-1'), 7.79
(s, 1 H, H-8).

10

Example 74

This example describes the synthesis of 1,3-di-n-
hexylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside.

A solution of 1,3-di-n-hexylxanthine-7-β-D-
15   ribofuranoside (0.17 g, 0.37 mmol), (1R)-(-)-
camphorsulphonic acid (0.088 g, 0.378 mmol) in dry
acetone (5 ml) was stirred for 20 h at room temperature.
The solvent was removed by rotary evaporation and the
residue was purified by silica gel column chromatography
20   (chloroform-methanol, 20:1) to yield 1,3-di-n-
hexylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside
(0.132 g, 70%) as a thick syrup. ^{1}H NMR (CDCl$_3$) d 0.88
(m, 6 H, 2 x CH$_3$), 1.26-1.38 (m, 15 H, 6 x CH$_2$ and
isopropylidene), 1.61-1.69 (m, 5 H, CH$_2$ and
25   isopropylidene), 1.72-1.76 (m, 2 H, CH$_2$), 3.83-4.02 (m, 5
H, H-4', 5' and N-CH$_2$), 4.11 (pseudo t, J = 7.7 and 7.3
Hz, 2 H, N-CH$_2$), 4.34 (br s, 1 H), 5.10 (dd, J = 6.8 and
4.9 Hz, 1 H), 5.22 (dd, J = 6.9 and 3.2 Hz, 1 H), 5.74
(d, J = 4.8 Hz, 1 H, H-1'), 7.78 (s, 1 H, H-8).

30

Example 75

This example describes the synthesis of methyl 1,3-
di-n-butylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuronate.

35   A solution of sodium periodate (1.61 g, 7.53 mmol)
in water (15 ml) was added to a solution of 1,3-di-n-

129

butylxanthine-7-β-D-2,3-O-isopropylidene-ribofuranoside
(0.8 g, 1.83 mmol) in chloroform:acetonitrile(1:1, 20 ml)
at room temperature and followed by ruthenium (III)
chloride (3.8 mg, 0.018 mmol). The reaction mixture was
5   vigorously stirred for 6 days at room temperature. After
the two layers were separated, aqueous layer was
extracted with chloroform (2 x 50 ml), and the combined
organic layer was washed with brine, dried over anhydrous
MgSO$_4$, filtered, and concentrated to dryness to yield
10   crude 1,3-di-n-butylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuronic acid (0.74 g) as a foam.

       To a solution of acid (dried *in vacuo* for 3 h) in
methanol (15 ml) were added DMAP (0.02 g, 0.16 mmol) and
then EDAC (0.79 g, 4.13 mmol), and the reaction mixture
15   was stirred for 17 h at room temperature. After removal
of solvent, the residue was dissolved in ethyl acetate
(70 ml) and washed with water (2 x 30 ml), and brine (40
ml), dried over anhydrous MgSO$_4$, filtered, and
concentrated to dryness. The residue was purified by
20   silica gel column chromatography (hexanes-ethyl acetate,
1:1) to yield methyl 1,3-di-n-butylxanthine-7-β-D-2,3-O-
isopropylidene-ribofuronate (0.36 g, 47.2%) as a
colorless solid, and unreacted 1,3-di-n-butylxanthine-7-
β-D-2,3-O-isopropylidene-ribofuranoside (0.24 g) was
25   recovered. ^{1}H NMR (CDCl$_3$) d 0.92-0.99 (m, 6 H, 2 x CH$_3$),
1.32-1.47 (m, 7 H, 2 x CH$_2$ and isopropylidene), 1.55-1.69
(m, 5 H, CH$_2$ and isopropylidene), 1.71-1.79 (m, 2 H, CH$_2$),
3.77 (s, 3 H, -OCH$_3$), 3.95 (t, J = 7.8 Hz, 2 H, N-CH$_2$),
4.10 (pseudo t, J = 7.6 and 7.3 Hz, 2 H, N-CH$_2$), 4.80 (s,
30   1 H), 5.17 (d, J = 6.2 Hz, 1 H), 5.46 (d, J = 6.2 Hz, 1
H), 6.26 (s, 1 H, H-1'), 7.88 (s, 1 H, H-8).

                            Example 76
       This example describes the synthesis of methyl 1,3-
35   di-n-pentylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuronate.

130

A mixture of 1,3-di-*n*-pentylxanthine-7-β-D-2,3-O-
isopropylidene-ribofuranoside (136.7 mg, 0.29 mmol),
sodium periodate (258 mg, 1.21 mmol), and ruthenium (III)
chloride (0.8 mg, 0.0039 mmol) in
5   chloroform:acetonitrile:water (2:2:3, 7 ml) was
vigorously stirred for 86 h at room temperature. A
workup similar to that for the compound of Example 75
yielded crude 1,3-di-*n*-hexylxanthine-7-β-D-2,3-O-
isopropylidene-ribofuronic acid (109 mg) as thick syrup.

10   To a solution of acid in methanol (10 ml) were added
DMAP (2.8 mg, 0.023 mmol) and then EDAC (109 mg, 0.57
mmol), and the reaction mixture was stirred for 25 h at
room temperature. After removal of solvent, the residue
was dissolved in ethyl acetate (50 ml) and washed with
15   water (2 x 20 ml), and brine (20 ml), dried over
anhydrous $MgSO_4$, filtered, and concentrated to dryness.
The residue was purified by silica gel column
chromatography (hexanes-ethyl acetate, 1:1) to yield
methyl 1,3-di-*n*-pentylxanthine-7-β-D-2,3-O-
20   isopropylidene-ribofuronate (85 mg, 76%) as foam, and
unreacted 1,3-di-*n*-pentylxanthine-7-β-D-2,3-O-
isopropylidene-ribofuranoside (40 mg) was recovered. [1]H
NMR (DMSO-d_6) d 0.88 (m, 6 H, 2 x C$\underline{H}_3$), 1.26-1.46 (m, 11
H, 4 x C$\underline{H}_2$ and isopropylidene), 1.49-1.67 (m, 5 H, C$\underline{H}_2$ and
25   isopropylidene), 1.70-1.80 (m, 2 H, C$\underline{H}_2$), 3.77 (s, 3 H, -
OC$\underline{H}_3$), 3.94 (t, *J* = 7.6 Hz, 2 H, N-C$\underline{H}_2$), 4.09 (t, *J* =
7.5Hz, 2 H, N-C$\underline{H}_2$), 4.80 (s, 1 H), 5.16 (d, *J* = 6.1 Hz, 1
H), 5.45 (d, *J* = 6.5 Hz, 1 H), 6.25 (s, 1 H, H-1'), 7.87
(s, 1 H, H-8).

30

Example 77

This example describes the synthesis of methyl 1,3-
di-*n*-hexylxanthine-7-β-D-2,3-O-isopropylidene-
ribofuronate.

35   A mixture of 1,3-di-*n*-hexylxanthine-7-β-D-2,3-O-
isopropylidene-ribofuranoside (113 mg, 0.23 mmol), sodium

131

periodate (201 mg, 0.94 mmol), and ruthenium (III) chloride (1.23 mg, 0.006 mmol) in chloroform:acetonitrile:water (2:2:3, 7 ml) was vigorously stirred for 25 h at room temperature. A
5   workup similar to that for the compound of Example 75 yielded crude 1,3-di-n-hexylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronie acid (115 mg) as thick syrup.

To a solution of acid in methanol (2.3 ml) were added DMAP (2.8 mg, 0.023 mmol) and then EDAC (109 mg,
10 0.57 mmol), and the reaction mixture was stirred for 24 h at room temperature. After removal of solvent, the residue was dissolved in chloroform (50 ml) and washed with water (2 x 20 ml), and brine (20 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness.
15 The residue was purified by silica gel column chromatography (hexanes-ethyl acetate, 1:1) to yield methyl 1,3-di-n-hexylxanthine-7-β-D-2,3-O-isopropylidene-ribofuronate (65.1 mg, 55%) as a colorless solid, and unreacted 1,3-di-n-hexylxanthine-7-β-D-2,3-O-
20 isopropylidene-ribofuranoside (26.6 mg) was recovered.
^{1}H NMR (DMSO-d_6) d 0.88 (pseudo t, J = 6.6 and 6.4 Hz, 6 H, 2 x C$\underline{H}_3$), 1.22-1.49 (m, 15 H, 6 x C$\underline{H}_2$ and isopropylidene), 1.54-1.65 (m, 5 H, C$\underline{H}_2$ and isopropylidene), 1.69-1.77 (m, 2 H, C$\underline{H}_2$), 3.88 (s, 3 H, -
25 OC$\underline{H}_3$), 3.94 (pseudo t, J = 8.0 and 7.6 Hz, 2 H, N-C$\underline{H}_2$), 4.09 (pseudo t, J = 7.8 and 7.6 Hz, 2 H, N-C$\underline{H}_2$), 4.80 (s, 1 H), 5.17 (d, J = 6.0 Hz, 1 H), 5.46 (d, J = 6.5 Hz, 1 H), 6.26 (d, J = 2.2 Hz, 1 H, H-1'), 7.87 (s, 1 H, H-8).

30   Example 78
This example describes a radioligand binding assay used to study the structure activity relationship (SAR) at the A_3 receptor in a manner substantially similar to that set forth in Example 57.
35   The CHO cells stably expressing the A_3 receptor were grown in F-12 medium containing 10% FBS and

132

penicillin/streptomycin (100 U/ml and 100 μg/ml
respectively) at 37 °C in a 5% CO_2 atmosphere, and
membrane homogenates were prepared as described in van
Galen et al., Mol. Pharmacol., 45, 1101-1111 (1994), van

5   Bergen et al., ACS 206th National Meeting, Chicago, IL,
Abstract MEDI217 (August 1993), Gallo-Rodriguez et al.,
J. Med. Chem., 37, 636-646 (1994), Olah et al., Mol.
Pharmacol., 45, 978-982 (1994). The binding of [^{125}I]4-
amino-3-iodobenzyladenosine-5'-N-methyluronamide

10   ([^{125}I]AB-MECA) to the CHO cells membranes was performed
essentially as described in Gallo-Rodriguez et al., J.
Med. Chem., 37, 636-646 (1994); Olah et al., supra.
Assays were performed in 50/10/1 buffer in glass tubes
and contained 100 μl of the membrane suspension, 50 μl of

15   [^{125}I]AB-MECA (final concentration 0.3 nM) and 50 μl of
inhibitor. Inhibitors were routinely dissolved in DMSO
and were then diluted with buffer; final DMSO
concentrations never exceeded 1%. Incubations were
carried out in duplicate for 1 h at 37 °C and were

20   terminated by rapid filtration over Whatman GF/B filters,
using a Brandell cell harvester (Brandell, Gaithersburg,
MD). Tubes were washed three times with 3 ml of buffer.
Radioactivity was determined in a Beckman gamma 5500B g-
counter. Non-specific binding was determined in the

25   presence of 40 μM R-PIA. K_i-values were calculated
according to Cheng-Prusoff (Cheng et al., supra),assuming
a K_d for [^{125}I]AB-MECA of 1.48 nM (Fozard et al., Br. J.
Pharmacol., 109, 3-5 (1993).
        Binding of [^{3}H]PIA to A_1 receptors from rat brain

30   membranes and of [^{3}H]CGS 21680 to A_2 receptors from rat
striatal membranes was performed as described previously
(Schwabe et al., Naunyn-Schmiedeberg's Arch. Pharmacol.,
313, 179-187 (1980); Jarvis et al., J. Pharmacol. Exp.
Therap., 251, 888-893 (1989)). Adenosine deaminase (3

35   U/ml) was present during the preparation of brain
membranes, in which an incubation at 30 °C for 30 min is

133

carried out, and during the incubation with radioligand.
At least six different concentrations spanning three
orders of magnitude, adjusted appropriately for the IC_{50}
of each compound, were used. IC_{50} values, computer-
generated using a non-linear regression formula on the
InPlot program (GraphPAD, San Diego CA), were converted
to apparent K_i values using K_d values of 1.0 and 14 nM for
$[^3H]$ PIA and $[^3H]$ CGS 21680 binding, respectively, and the
Cheng-Prusoff equation (Cheng et al., supra).

Table 4: Affinities of xanthine riboside derivatives in radioligand binding assays at rat brain A_1, A_2, and A_3 receptors[a]

Compound	$K_i(A_1)$[b]	$K_i(A_2)$[c]	$K_i(A_3)$[d]	A_1/A_3	A_2/A_3
theophylline-7-riboside	27,000	2% (10^{-4})	89,400	0.30	>1
8-methoxy-theophylline-7-β-D-ribofuranoside [Example 59]	21 ± 3% (10^{-4})	4% (10^{-4})	74.0 ± 6.8	>1	>1
1,3-di-n-propylxanthine-7-β-D-ribofuranoside	15,900	32% (10^{-4})	81,200	0.20	>1
1,3-di-n-butylxanthine-7-β-D-ribofuranoside [Example 60]	4190	19,500	6030	0.69	3.2
1-benzyl-3-butylxanthine-7-β-D-ribofuranoside [Example 61]	2190 ± 390	8670 ± 1890	12,900 ± 300	0.17	0.67
3-benzyl-1-butylxanthine-7-β-D-ribofuranoside [Example 61]	1720 ± 30	4510 ± 1230	12,400 ± 20	0.14	0.36
1,3-di-n-butyl-2-thio-xanthine-7-β-D-ribofuranoside [Example 62]	2250 ± 470	4250 ± 580	1400 ± 160	1.6	3.0
1,3-di-n-pentylxanthine-7-β-D-ribofuranoside [Example 63]	8790 ± 1580	47,300 ± 2200	4810 ± 1070	1.8	9.8
1,3-di-n-hexylxanthine-7-β-D-ribofuranoside [Example 64]	9750 ± 400	7.8 ± 3.1% (10^{-5}), 69 ± 2% (10^{-4})	43,000 ± 3400	0.23	---
methyl 1,3-di-n-butylxanthine-7-β-D-ribofuronate [Example 65]	3720 ± 200	0% (3X10^{-6})	3230 ± 590	1.2	>1

Table 4: (Continued)

Compound	$K_i(A_1)^b$	$K_i(A_2)^c$	$K_i(A_3)^d$	A_1/A_3	A_2/A_3
1,3-di-n-butylxanthine-7-β-D-ribofuronamide [Example 66]	3060 ± 150	54,900 ± 5300	15,300 ± 700	0.20	3.6
N-methyl 1,3-di-n-butylxanthine-7-β-D-ribofuronamide [Example 67]	37,300 ± 4600	19 ± 2% (10^{-4})	229 ± 27	160	>400
N-methyl 1,3-di-n-pentylxanthine-7-β-D-ribofuronamide [Example 68]	11,200 ± 1000	2% (10^{-4})	2630 ± 260	4.3	>>1
N-methyl 1,3-di-n-hexylxanthine-7-β-D-ribofuronamide [Example 69]	30,800 ± 4900	0% (10^{-4})	10,300 ± 360	3.0	>10
N,N-dimethyl 1,3-di-n-butylxanthine-7-β-D-ribofuronamide [Example 70]	9100 ± 2450	53,500 ± 8700	228,000^l ± 9000	0.040	0.23
N-ethyl 1,3-di-n-butylxanthine-7-β-D-ribofuronamide [Example 71]	6210 ± 1060	38,100 ± 3500	602 ± 76	10	63

a A percent value indicates the percent displacement of radioligand at the concentration (M) given in parentheses.
b Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes, expressed as K_i ± S.E.M. in nM (n = 3-5).
c Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± S.E.M. in nM (n = 3-6).
d Displacement of specific [^{125}I]AB-MECA binding, unless noted, in membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± S.E.M. in nM (n = 3-5).

136

As is apparent from the results set forth in Table
4, theophylline-7-riboside and the corresponding 1,3-
dipropyl and dibutyl analogues bind weakly to A_3
receptors. These results clearly define the dependence
of affinity of xanthine-7-ribosides at all of the
adenosine receptors on the size of the N_3 and N_1 alkyl
substituents. Affinity at A_3 receptors was enhanced by
1,3-dialkyl substituents in the order Pent $\geq$ Bu $>>$ Hx $>$
Pr $\approx$ Me. The rank order of affinity at A_1 receptors for
1,3-dialkyl substituents was: Bu $>$ Pent $\approx$ Hex $>$ Pr $>$ Me,
and, similarly, the order at A_{2a} receptors was: Bu $>$ Pent
$>$ Pr. In the ribose series, the 1,3-dipentyl analogue,
namely 1,3-di-n-pentylxanthine-7-β-D-ribofuranoside, had
the most favorable ratio of A_3 receptor selectivity,
although it was only slightly selective (2-fold vs. A_1
and 10-fold vs. A_{2a}).

The introduction of an aromatic group on the 1- or
3-substituent, as in the benzyl derivatives 1-benzyl-3-
butylxanthine-7-β-D-ribofuranoside and 3-benzyl-1-
butylxanthine-7-β-D-ribofuranoside tended to increase the
potency slightly at A_1 and A_{2a} receptors (2-4 fold) and
decrease potency slightly at A_3 receptors (2-fold)
relative to 1,3-di-n-butylxanthine-7-β-D-ribofuranoside.
Thus, it appears that, in the ribose series, the presence
of a benzyl group is somewhat disadvantageous for
achieving selectivity for A_3 receptors.

The introduction of 8-substitution theophylline-7-
riboside in the form of a methoxy group (8-methoxy-
theophylline-7-β-D-ribofuranoside) was found to be
detrimental to affinity at A_1 receptors but not at A_3
receptors. Thus, although a very weak competitor of
binding, 8-methoxy-theophylline-7-β-D-ribofuranoside
appears to be slightly selective for A_3 receptors.

The presence of a 2-thio group vs. a 2-oxo group in
the pair of 1,3-dibutylxanthine-7-ribosides (cf. 1,3-di-
n-butylxanthine-7-β-D-ribofuranoside vs. 1,3-di-n-butyl-

137

2-thio-xanthine-7-β-D-ribofuranoside) increased potency
at all three subtypes (4-fold at A_3 receptors) and
slightly increased A_3 vs. A_1 selectivity.

   The introduction of an ester group at the 5'-
5   position (cf. methyl 1,3-di-n-butylxanthine-7-β-D-
ribofuronate vs. 1,3-di-n-butylxanthine-7-β-D-
ribofuranoside) maintained the same affinity at A_1
receptors as the corresponding 5'-CH$_2$OH analogue and
increased affinity at A_3 receptors by 2-fold. Thus,
10   methyl 1,3-di-n-butylxanthine-7-β-D-ribofuronate is of
equal affinity at A_1 and A_3 receptors, but is selective
for A_3 vs. A_{2a} receptors. Like the ester derivative, the
5'-primary carboxamide analogue 1,3-di-n-butylxanthine-7-
β-D-ribofuronamide maintained roughly the same affinity
15   at A_1 receptors vs. 1,3-di-n-butylxanthine-7-β-D-
ribofuranoside. However, 1,3-di-n-butylxanthine-7-β-D-
ribofuronamide displayed a decreased affinity at A_{2a} and
A_3 receptors by 2.8- and 2.5-fold, respectively, vs. 1,3-
di-n-butylxanthine-7-β-D-ribofuranoside.

20   The 5'-uronamide modification, which was previously
found to result in A_3 selectivity in N^6-benzyl adenosine
derivatives, was incorporated into xanthine-7-ribosides.
The affinity of 5'-uronamides at A_3 receptors depended on
the N-alkyluronamide substituent in the order MeNH > EtNH
25   >> NH$_2$ >> Me$_2$N (cf. the 1,3-dibutylxanthine analogues of
Examples 66, 67, and 69-71). 1,3-Dibutylxanthine-7-
riboside-5'-N-methyl-carboxamide, with a K_i value of 229
nM at A_3 receptors, was 160-fold selective for A_3 vs. A_1
receptors and >400-fold selective vs. A_{2a} receptors. The
30   presence of the N-methyl group vs. the primary
carboxamide, 1,3-di-n-butylxanthine-7-β-D-ribofuronamide,
caused a 67-fold increase in affinity at A_3 receptors,
and at both A_1 receptors or A_{2a} receptors affinity
diminished. Multiple substitution of the 5'-carboxamide
35   group as in the N,N-dimethyl analogue, N,N-dimethyl 1,3-
di-n-butylxanthine-7-β-D-ribofuronamide, was not

138

favorable for A_3 selectivity. Relative to N-methyl 1,3-
di-n-butylxanthine-7-β-D-ribofuronamide, this resulted in
slightly increased affinity at A_1 and A_{2a} receptors, and
affinity at A_3 receptors was decreased by 1000-fold. The

5   N-ethyl analogue, N-ethyl 1,3-di-n-butylxanthine-7-β-D-
ribofuronamide, was slightly less potent than N-methyl
1,3-di-n-butylxanthine-7-β-D-ribofuronamide at A_3
receptors, but less selective. Thus, a monomethyl
substitution in the 5'-uronamide series appears to be

10   optimal.

Thus, the effect of decreased A_3 potency upon
increase in the size of the purine 1- and 3-alkyl groups
from butyl to hexyl, noted above for the 7-riboside
series, was not seen in the 5'-uronamide series. The

15   affinity of the 5'-uronamides was dependent on the 1,3-
dialkyl substitution in the order Bu > Pent > Hex.

Example 79

This example illustrates other possible substituents
20   which can be used in conjunction with the present
inventive compounds and those compounds useful in the
context of the present inventive method.

The following three compounds were synthesized and
then their A_3-affinities were determined.
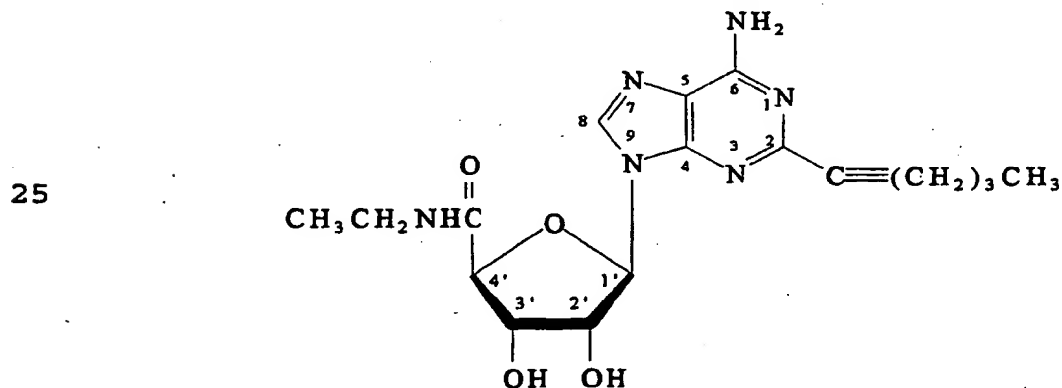
139
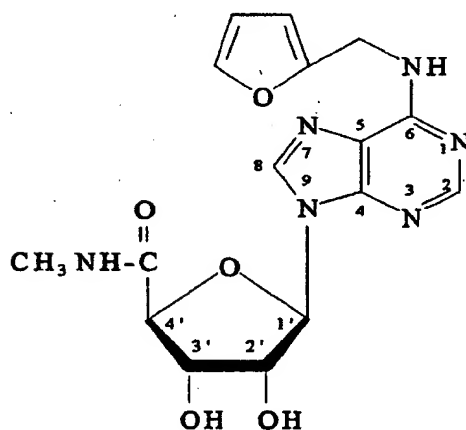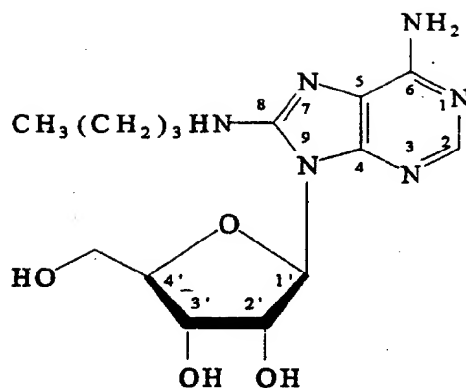
The K_i's (nM) at the A_3 receptors for these three compounds were 25.6 ± 3.2, $100,000$, and 720 ± 250, respectively. The heterocylic aryl substituent in the last of the three compounds, in particular, demonstrates that a wide variety of substituent groups may be utilized in conjunction with the present inventive compounds and those compounds useful in the context of the present inventive method without detracting from the high affinities of such compounds.

In addition, an isothiocyanate group in the 3-position of the N^6-benzyl ring provides a means for covalently linking the A_3 agonist to its receptor. N^6-(3-isothiocyanatobenzyl)adenosine-5'-N-methyluronamide was

140

found to bind selectively to A_3 receptors. K_i values for
this isothiocyanate derivative in competition binding at
rat brain A_1, A_{2a}, and A_3 receptors were 145, 272, and
10.0 nM, respectively. A preincubation with this
5   derivative resulted in irreversible inhibition of
radioligand binding at rat A_3 receptors in membranes of
transfected CHO cells or RBL-2H3 mast cells, but not at
rat A_1 or A_{2a} receptors. The loss of binding sites for
0.1 nM $[^{125}I]N^6$-(4-aminobenzyl)adenosine-5'-N-
10   methyluronamide, a high affinity A_3 receptor radioligand,
in transfected CHO cell membranes was concentration-
dependent with an IC_{50} of 50 nM. No change was observed
in the K_d value of the remaining A_3 receptor sites. The
inhibition was also insensitive to theophylline (1 mM),
15   consistent with the pharmacology of rat A_3 receptors.
Structurally similar adenosine analogues lacking the
chemically reactive isothiocyanate group failed to
irreversibly inhibit A_3-binding.

      Moreover, primary amino derivatives served as
20   functionalized congeners for coupling to other moieties
for detection (by virtue of a radioactive or fluorescent
or other spectroscopic label) or targeting purposes,
without loss of affinity or A_3 selectivity. The
attachment points for the functionalized chains were at
25   the 2-position of the adenine ring and at the 3-position
of the benzyl ring. Examples of cogeners are: 2-chloro-
N^6-[3-(2-aminoethylcarbonylamino)-benzyl]adenosine-5'-N-
methyluronamide, 2-(1-hexynyl)-N^6-[3-(2-
aminoethylcarbonylamino)benzyl]adenosine-5'-N-
30   methyluronamide, and 2-(6-amino-1-hexynyl)-N^6-(3-
iodobenzyl)adenosine-5'-N-methyluronamide. Amides or
thiourea groups were typically formed, using active
esters, isothiocyanate, etc. derivatives of the added
moiety.

35

141

Example 80

This example demonstrates the cerebral protective effect, particularly as to spontaneous seizures, resulting from the administration of an A_3-selective adenosine receptor agonist.

Spontaneous seizures are commonly observed in gerbils. Their presence constitutes a part of the normal, defensive behaviour in these animals. Hence, in gerbils, convulsive activity is unrelated to genetic modifications introduced artificially (e.g., laboratory bred, seizure-prone mice or rats). Spontaneous seizures are observed in 50-60% of the wild gerbil population. Seizures are easily elicited by either an unfamiliar or potentially threatening stimulus or environment.

During the studies of behavioural responses of gerbils to adenosine receptor stimulation, animals were exposed to the water maze model of spatial learning and memory. The control group (n = 15) was injected with the vehicle given i.p. for 6 weeks prior to water maze tests. Twenty-four hours after the last injection, animals were exposed to the swim task. Immersion of animals in the swim tank whose water was kept at 32°C produced an immediate, long-lasting (up to 2 min) seizure complex that was presented as myoclonic convulsions, followed by a period of rigidity and hind-limb extension. Convulsions were observed in 60% of the control group and disappeared within 4-5 days following the first immersion, i.e., the period required for animals to acclimatize to the new environment.

Contrary to the controls, chronic treatment of gerbils (n = 20) with adenosine A_3 receptor IB-MECA (100 μg/kg i.p. daily for 6 wks) administered prior to water exposure during the swim task entirely eliminated seizing activity both during the first and subsequent water exposures. The acute administration of IB-MECA, however, was found to worsen the seizing activity.

142

In view of these data, it is clear that the chronic administration of IB-MECA and related adenosine A_3 receptor agonists is effective as an antiepileptogenic treatment in the context of both artificially induced
5   seizures and spontaneous epileptic activity as seen in humans, and has, therefore, a significant therapeutic value.

Example 81

10   This example demonstrates the cerebral protective effect, particularly as to seizures induced by NMDA, pentamethylenetetrazole, and electric shock, resulting from the administration of an A_3-selective adenosine receptor agonist.

15   In gerbils, preischemic stimulation of adenosine A_3 receptors with a selective adenosine A_3 receptor agonist, particularly, N^6-(3-iodobenzyl) adenosine-5'-N-methylcarboxamide (IB-MECA), produces a regimen dependent effect, i.e., acute administration worsens, while chronic
20   treatment improves, the outcome of brain ischemia of moderate intensity. Since several pathophysiological processes (e.g., activation of phospholipases A_2 and C, formation of IP_3) are typical of both cerebral ischemia and seizures, the effect of acute and chronic
25   administration of IB-MECA on the outcome of seizures was further investigated. The effects of IB-MECA were studied in models in which seizures were elicited using different mechanisms, i.e., neuronal hyperactivation [N-methyl-D-aspartate (NMDA)-evoked seizures], perturbation
30   of GABA-ergic inhibition [pentamethylenetetrazole-induced convulsions (Rehavi et al., Eur. J. Pharmacol., 78, 353 (1978)], and generalized convulsions [electric shock (Mastropaolo et al., Pharmacol. Biochem. Behav., 41, 663 (1992)].

35   Male C57Bl/J5 mice (Jackson Laboratory, Bar Harbor, Maine) weighing 35 g were used in this study. All drugs

143

were injected i.p. using a 25 gauge hypodermic needle.
The adenosine A_3 receptor agonist N^6-3-(iodobenzyl)
adenosine-5'-N-methylcarboxamide (IB-MECA) was dissolved
in a 20:80 v/v solution of Alkamuls 620 (Rhône-Poulenc,
5   Cranbury, N.J.) and injected either acutely or
chronically. In the acute regimen, the drug was given at
10, 50, or 100 µg/kg. After establishing the dose
response for chemically induced seizures in the acute
regimen, the dose characterized by highest efficacy in
10 all studied measures (i.e., 100 µg/kg) was selected for
acute administration in studies of electroconvulsive
shock. The same dose was selected for chronic
administration (daily injections for 6 weeks). Saline-
dissolved N-methyl-D-aspartate [NMDA (Research
15 Biochemicals International, Natick, MA)] was given
acutely at either 60 or 125 mg/kg, while acute
injections of saline-dissolved pentamethylenetetrazole
(Aldrich, Milwaukee, WI) were given at 75 mg/kg.
          Previous experiments showed no statistical
20 differences in the response to chemoconvulsants in
animals injected either acutely or chronically with the
vehicle. Therefore, in the present study, the
chemoconvulsant controls were injected with the vehicle
following the chronic schedule only.
25   All convulsant control and IB-MECA groups consisted
of 10-15 animals. In the acute regimen, IB-MECA was
injected 15 min prior to the administration of NMDA or
pentamethylenetetrazole. In the chronic regimen,
convulsants were administered 24 h after the last
30 injection of IB-MECA. Injections of the vehicle followed
the same pattern.
          Graded electric shocks lasting 0.3 s were generated
by a Hittman electroconvulsive shock generator (Modcraft
mod. B24-III) and administered via ear clip electrodes.
35 Starting from 70 V, shocks were increased every 2 s in 10
V steps until either a full tonic seizure was elicited or

144

a maximum voltage of 170 V was reached. In the electric
shock experiments, injections of IB-MECA were performed
using acute or chronic regimen described above. Controls
were injected with the vehicle following either the acute
5   or chronic schedule of IB-MECA administration.

All experiments were performed in a quiet room
illuminated with low intensity fluorescent lighting.
Following injection of the convulsant or administration
of electric shocks, mice were placed in individual
10   transparent observation cages. In chemically-induced
seizures, the onset, duration, and intensity of the
subsequent neurological symptoms were monitored for the
following 15 min. Thereafter, animals were transferred
to their home cages, and their behaviour and mortality
15   were observed for the subsequent 24 h. Since electric
shock elicits an "either-or" response, only the tonic
seizure-producing voltage and survival during the
subsequent 24 h were observed in this part of the study.

The temperature effect of IB-MECA was measured using
20   the Harvard (South Natick, MA) rectal probe in animals
subjected to a light halothane anesthesia. The
temperature was measured either 15 min after acute
administration of 100 μg/kg IB-MECA or 24 h after the
last injection of the drug when given chronically
25   (N=5/group). There were no significant temperature
differences following either acute or chronic
administration of the drug.

The results of the NMDA experiments are set forth in
Table 5 below, wherein the following neurological
30   impairment scale was utilized: 1 - no change, 2 -
depression, 3 - scratching/biting, 4 - locomotor
hyperactivity, 5 - clonic seizures, 6 - clonic/tonic
complexes. The results of the PMT and electroshock
experiments are set forth in Tables 6 and 7,
35   respectively.

145

Table 5: Percentage of animals with convulsive behavior appearing within 15 min of administration of NMDA and neurological impairment and mortality after administration of NMDA

Compound	% Convulsing (P)[b]	Ave. Latency to Seizures (sec + S.E.M.) (P)[c]	Impairment + S.E.M. (P)[d]	% Mortality at 24 h (P)[e]
NMDA (60 mg/kg)[a]	80	244 + 90	3.5 + 0.5	60
IB-MECA (10 μg/kg) + NMDA (60 mg/kg)	50 (NS)	581 + 107 (<0.05)	2.7 + 0.6 (NS)	50 (NS)
IB-MECA (50 μg/kg) + NMDA (60 mg/kg)	10 (<0.05)	12[f] (NA)	2.0 + 0.5 (NS)	27 (NS)
acute IB-MECA (100 μg/kg) + NMDA (60 mg/kg)	0 (NA)	NA (NA)	1.4 + 0.4 (<0.05)	10 (<0.05)
chronic IB-MECA (100 μg/kg) + NMDA (60 mg/kg)	30 (<0.05)	689 + 108 (<0.05)	1.8 + 0.4 (<0.05)	20 (<0.05)

[a] NMDA control group: N=15; all other groups: N=10
[b],[e] compared to NMDA control group; Fisher's test with Bonferroni's correction
[c],[d] compared to NMDA control group; Dunnett's test
[f] only one animal
NA = not applicable
NS = not significant

Table 6: Onset of convulsive behavior, degree of neurological impairment, and mortality in animals treated with PMT

Compound	Onset (sec) + S.E.M.	Impairment + S.E.M.	Mortality (%)				
			<15 min	0.4-5 h	>5 h	Overal l	P[b]
PMT (75 mg/kg)	74 + 12	5.0 % 0.5	100	0	0	100	
acute IB-MECA (100 μg/kg) + PMT (75 mg/kg)	112 + 10[a]	4.1 + 0.5	40	0	10	50	<0.05
chronic IB-MECA (100 μg/kg) + PMT (75 mg/kg)	120 + 9	3.3 + 0.5[a]	40	10	0	50	<0.05

[a] P < 0.05; Student-Newman-Keuls test
[b] Fisher's exact test with Bonferroni's correction
PMT = pentamethylenetetrazole

146

Table 7: Threshold voltage and mortality of animals subjected to electroshock

Compound	Threshold Voltage + S.E.M.	% Mortality (at 24 h)
acute vehicle + E.S.	91 + 4	80
acute IB-MECA + E.S.	106 + 10	70
chronic vehicle + E.S.	100 + 11	80
chronic IB-MECA + E.S.	118 + 13	30 (p < 0.05)[a]

[a] compared to chronic vehicle + E.S.; Fisher's test
E.S. = electric shock

A.   Acute Experiments

As is apparent from the results of Table 5, when compared to NMDA alone, acute administration of IB-MECA at 10 μg/kg prior to 60 mg/kg NMDA had no effect on either the incidence of seizures, or the degree of neurological impairment. However, administration of IB-MECA at 10 μg/kg caused a small but significant delay in the onset of convulsive behaviour. In the group given IB-MECA at 50 μg/kg, seizures within the initial 15 min after administration of NMDA were present in only one animal, while a long lasting locomotor depression characterized the rest of the group. Persistent myoclonic jerks were subsequently observed in two animals at ~ 30 to 45 min following the NMDA injection.

In the 10 μg/kg IB-MECA group, the mortality approached very closely that attained with NMDA alone, and all deaths occurred within 15 min following administration of NMDA. However, in the 50 μg/kg IB-MECA group only one animal died within that time (12 sec). The two subsequent deaths in that group (animals with myoclonic jerks) occurred at ~ 1 h after injection of NMDA.

At 100 μg/kg IB-MECA was protective in all studied measures. Although occasional scratching was present in some animals, clinically manifested seizures were completely absent. Neurological disturbances

147

accompanying acute IB-MECA at 100 μg/kg followed by NMDA
at 60 mg/kg were limited to a period of lethargic
behaviour lasting 45-60 min after the injection of NMDA.
During this period animals were fully responsive to
5   external stimuli (sound, touch). A noxious stimulus
(touch or tap with the tip of a pencil) caused a rapid
translocation followed by a renewed period of locomotor
quiescence. While moving, the gait of all animals
appeared to be fully normal. The mortality was 10%, with
10   the solitary death occurring 25 min after administration
of NMDA.

Administration of pentamethylenetetrazole alone
resulted in the death of all animals within 15 min. When
IB-MECA preceded the convulsant, the onset of seizures
15   and overall mortality were significantly reduced. There
was no effect on the neurological impairment (Table 6).

Acute administration of IB-MECA resulted in an
insignificant increase of threshold voltage (Table 7).
Mortality remained unaffected.
20

B.   Chronic Experiments
Chronic administration of IB-MECA resulted in the
reduction of neurological impairment (Table 5). Tonic
seizures were observed in 20% of animals, but their onset
25   was significantly delayed. In the remaining animals,
injection of NMDA rapidly elicited a protracted period of
lethargic behaviour similar to that seen in animals
injected acutely with IB-MECA at 100 μg/kg followed by 60
mg/kg NMDA. Following chronic administration of IB-MECA,
30   the mortality was reduced to 30% (Table 5).

Chronic injection of IB-MECA prior to 75 mg/kg
pentamethylenetetrazole had no significant effect on
either the onset of seizures or neurological impairment
(Table 6); however, the mortality was significantly
35   reduced. The reduction did not differ from that seen
following acute administration of IB-MECA.

148

Chronic treatment with IB-MECA produced no significant changes in threshold voltage; however, postictal mortality was significantly reduced (Table 7).

5   Example 82

This example demonstrates the anti-inflammatory effect resulting from the administration of an A_3-selective adenosine receptor agonist.

It is known that acute administration of IB-MECA
10   results in stimulation of phospholipase C and an enhanced production of inositol 1,4,5-triphosphate. Similar stimulation characterizes ischemic and inflammatory processes in the brain, heart, and majority of other tissues. It is also known that following their
15   initiation, these processes lead, in turn, to the activation of phospholipase A_2, and to subsequent arachidonic acid cascades, release of harmful prostanoid species, and production of free radicals. As a result of direct tissue impact (i.e., damage to cell membranes and
20   organelles), and of prostanoid-mediated compromise of regional microcirculation, inflammatory processes ensue (aseptic inflammation).

The acute administration of IB-MECA impairs recovery of postischemic blood flow, whereas chronic
25   administration of this drug improves it. Since microcirculatory compromise accompanies both inflammation and ischemia (following which inflammation has been also observed, cf. Greenfield's Neuropathology (Wiley & Sons)), chronically administered IB-MECA will act as an
30   antiinflammatory agent affecting and reducing the intensity of both cellular (stimulation of phospholipase C and indirect effect on phospholipase A_2) and tissue responses (amelioration of regional microcirculatory reaction) to injury. These properties of IB-MECA and
35   similar agents acting at adenosine A_2 receptors indicate their applicability as antiinflammatory therapeutics in

149

the treatment of aseptic and, possibly, also septic
tissue inflammation and trauma.

Example 83

5   This example demonstrates the procognitive effects
resulting from the administration of an A_3-selective
adenosine receptor agonist.

Studies of learning and memory were carried out in
gerbils chronically injected with IB-MECA (100 μg/kg,
10   daily for 2 months, n=20). These studies indicated that
IB-MECA treatment decreases the time required for
learning a task in the Morris water maze (a model of
spatial memory) and results in significantly improved
performance of the task (average time to target IB-MECA
15   group 30 sec vs. controls injected chronically with the
vehicle 40 sec, p<0.04). Open field studies of
locomotion did not show any significant differences in
locomotor behavior between animals injected chronically
with IB-MECA and animals injected chronically with the
20   vehicle.

Example 84

This example demonstrates the high correlation
between ligand affinity for A_3 adenosine receptors in
25   various species, particularly as between rats, gerbils,
and humans.

The binding affinities at A_3 adenosine receptors in
different species were compared by using the high
affinity A_3 adenosine agonist ligand [^{125}I]AB-MECA (3-
30   iodo-4-aminobenzyladenosine-5'-N-methyluronamide) in the
presence of 1.0 μM of the A_1- and A_{2a}-adenosine antagonist
XAC (8-[4-[[[[(2-
aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-
dipropylxanthine). XAC was added to eliminate binding to
35   non-A_3 receptors. In rat brain membranes [^{125}I]AB-MECA
exhibited saturable, specific binding with a K_d of 2.28

150

nM and a B_{max} of 43 fmol/mg protein. The affinity of $[^{125}I]$AB-MECA at the gerbil and rabbit brain A_3-receptors was similar to the rat, demonstrating that the affinity of this agonist is not species dependent.

5   Agonist affinities were measured at cloned rat brain A_3 receptors stably expressed in CHO cells. K_i values (expressed as nM vs. binding of $[^{125}I]$APNEA) were determined to be: S-PIA, 920 ± 311; CPA, 240 ± 36; R-PIA, 158 ± 52; and NECA, 113 ± 34. These values were

10   compared with competition data for agonists at the human brain A_3 receptors, also expressed in CHO cells (Salvatore et al., <u>Proc. Natl. Acad. Sci. USA</u>, <u>90</u>, 10365-10369 (1993)). A high degree of correlation was found as shown in Figure 8, which demonstrates that human and rat

15   brain A_3 receptors are highly similar in the relative affinity of agonists.

      Thus, among adenosine agonists of varied structure (e.g., 5'-, 2-, and N^6-derivatives), binding at rat A_3 receptors is similar in relative order to binding at

20   human A_3 receptors. Indeed, although the K_i values in rat were several-fold higher than those in human, the correlation between human and rat receptors was found to be linear for the tested adenosine derivatives (Figure 8, $r^2 = 0.995$).

25

      All of the references cited herein, including publications, patents, and patent applications, are hereby incorporated in their entireties by reference.

      While this invention has been described with an

30   emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this

35   invention includes all modifications encompassed within

the spirit and scope of the invention as defined by the
following claims.

152

<u>WHAT IS CLAIMED IS</u>:

1.   A compound of the formula
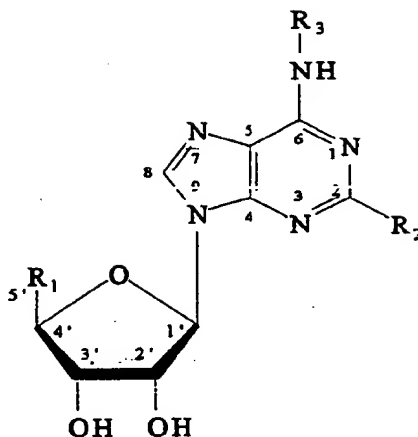
R_3
|
NH
N   5   6 1N
7
8   9
N   4   N   R_2

R_1   O
5'
4'   1'
3'   2'

OH   OH

5   wherein R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a and R^b may be
the same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
10   C_3-C_{10} cycloalkyl, or are joined together to form a
heterocyclic ring containing two to five carbon atoms,
and R^c is selected from the group consisting of C_1-C_{10}
alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10}
boc-aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is selected from
15   the group consisting of hydrogen, halo, C_1-C_{10}
alkylethers, amino, C_1-C_{10} alkylamino, C_2-C_{10} alkenes, C_2-
C_{10} alkynes, thio, and C_1-C_{10} alkylthio, and R_3 is
selected from the group consisting of R- and S- 1-
phenylethyl, an unsubstituted benzyl group, and a
20   phenylethyl or benzyl group substituted in one or more
positions with a substituent selected from the group
consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo.

25   2.   The compound of claim 1, wherein R_1 is
$R^aR^bNC(=O)$, wherein R^a and R^b may be the same or different
and are selected from the group consisting of hydrogen,

153

C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_2-C_{10} alkenes, and C_2-C_{10} alkynes, and R_3 is selected from

5   the group consisting of R- and S- 1-phenylethyl, an unsubstituted benzyl group, and a benzyl group substituted in one or more positions with a substituent selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10}

10   alkoxy, and sulfo.

3.   The compound of claim 2, wherein R_2 is hydrogen or halo.

15   4.   The compound of claim 2, wherein R^a is hydrogen and R_2 is hydrogen.

5.   The compound of claim 4, wherein R_3 is unsubstituted benzyl.

20

6.   The compound of claim 5, wherein R^b is a C_1-C_{10} alkyl or C_3-C_{10} cycloalkyl.

7.   The compound of claim 6, wherein R^b is a C_1-C_{10}

25   alkyl.

8.   The compound of claim 7, wherein R^b is methyl.

9.   The compound of claim 4, wherein R_3 is R-1-

30   phenylethyl, S-1-phenylethyl, or a substituted benzyl.

10.   The compound of claim 9, wherein R^b is a C_1-C_{10} alkyl or C_3-C_{10} cycloalkyl.

35   11.   The compound of claim 10, wherein R^b is a C_1-C_{10} alkyl.

154

12.   The compound of claim 11, wherein R^b is methyl
or ethyl.

13.   The compound of claim 12, wherein R_3 is a
substituted benzyl.

14.   The compound of claim 13, wherein R_3 is a
benzyl substituted in one or more positions with a
substituent selected from the group consisting of halo,
amino, acetamido, C_1-C_{10} haloalkyl, and sulfo.

15.   The compound of claim 14, wherein R_3 is a halo-
substituted benzyl.

16.   The compound of claim 15, wherein the compound
is N^6-3-iodobenzyl-5'-N-methylcarboxamidoadenosine.

17.   The compound of claim 2, wherein R^a and R^b may
be the same or different and are selected from the group
consisting of hydrogen and C_1-C_{10} alkyl.

18.   The compound of claim 1, wherein R_1 is
$R^aR^bNC(=O)$, wherein R^a and R^b may be the same or different
and are selected from the group consisting of hydrogen,
C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl,
and C_3-C_{10} cycloalkyl, R_2 is selected from the group
consisting of halo, C_1-C_{10} alkylethers, amino, C_1-C_{10}
alkylamino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, thio, and C_1-
C_{10} alkylthio, and R_3 is selected from the group
consisting of R- and S- 1-phenylethyl, an unsubstituted
benzyl group, and a benzyl group substituted in one or
more positions with a substituent selected from the group
consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo.

155

19. The compound of claim 18, wherein R^a is hydrogen and R_2 is halo, C_1-C_{10} alkylamino, or C_1-C_{10} alkylthio.

5   20. The compound of claim 19, wherein R_3 is a substituted benzyl.

21. The compound of claim 20, wherein R^b is a C_1-C_{10} alkyl.

10

22. The compound of claim 21, wherein said compound is selected from the group consisting of 2-chloro-N^6-(3-iodobenzyl)-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine, N^6-(3-iodobenzyl)-2-methylamino-9-[5-

15   (methylamido)-β-D-ribofuranosyl]-adenine, and N^6-(3-iodobenzyl)-2-methylthio-9-[5-(methylamido)-β-D-ribofuranosyl]-adenine.

23. The compound of claim 2, wherein R_2 is a C_2-C_{10}

20   alkyne of the formula R'-C≡C- where R' is a C_1-C_8 alkyl.

24. A compound selected from the group consisting of N^6-benzyladenosine-5'-N-alkyluronamide-N^1-oxide and N^6-benzyladenosine-5'-N-dialkyluronamide-N^1-oxide.

25

25. A compound of the formula
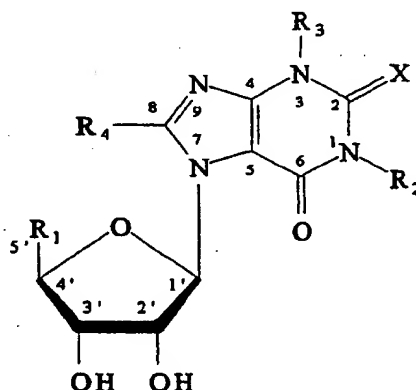
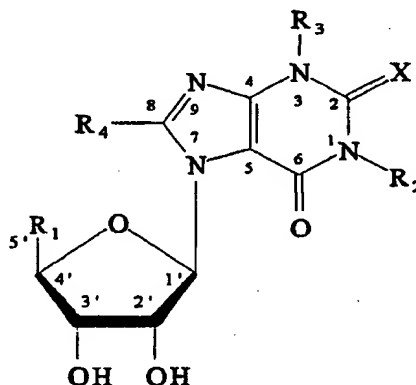wherein X is O or S, R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a
and R^b may be the same or different and are selected from
the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-
C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, or

5   are joined together to form a heterocyclic ring
containing two to five carbon atoms, and R^c is selected
from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
C_3-C_{10} cycloalkyl, R_2 and R_3 may be the same or different

10   and are selected from the group consisting of C_1-C_{10}
alkyl, R- and S- 1-phenylethyl, an unsubstituted benzyl
group, and a phenylether or benzyl group substituted in
one or more positions with a substituent selected from
the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10}

15   haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and
sulfo, and R_4 is selected from the group consisting of
halo, benzyl, phenyl, C_3-C_{10} cycloalkyl, and C_1-C_{10}
alkoxy, with the proviso that if X = O, R_1 is $HOCH_2$, and
R_4 = H, then R_2 and R_3 are not C_1-C_4 alkyl.

20

        26.   The compound of claim 25, wherein X is O, R_1 is
$R^aR^bNC(=O)$, wherein R^a and R^b may be the same or different
and are selected from the group consisting of hydrogen,
C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl,

25   and C_3-C_{10} cycloalkyl, R_2 and R_3 may be the same or
different and are selected from the group consisting of
C_1-C_{10} alkyl, R- and S- 1-phenylethyl, an unsubstituted
benzyl group, and a benzyl group substituted in one or
more positions with a substituent selected from the group

30   consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo, and
R_4 is selected from the group consisting of halo, benzyl,
phenyl, and C_3-C_{10} cycloalkyl.

157

27. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 1.

28. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 2.

29. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 24.

30. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 25.

31. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 26.

32. A method of selectively activating an A_3 adenosine receptor in a mammal, which method comprises administering to a mammal in need of selective activation of its A_3 adenosine receptor a therapeutically effective amount of a compound which binds with the A_3 receptor so as to stimulate an A_3 receptor-dependent response.

33. The method of claim 32, wherein said compound has the formula

158

R3
|
NH
|

(structure: purine-type ring system with positions labeled N5, 6, N1, 7, 8, 9, 4, 3, 2, N; R2 attached; attached to sugar ring with O, positions 5', R1, 4', 3', 2', 1'; OH OH)
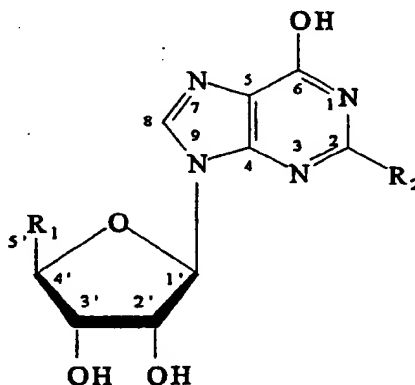
wherein R_1 is $R^a R^b NC(=O)$ or HOR^c, wherein R^a and R^b may be
the same or different and are selected from the group

5   consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}
haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and
C_3-C_{10} cycloalkyl, or are joined together to form a
heterocyclic ring containing two to five carbon atoms,
and R^c is selected from the group consisting of C_1-C_{10}

10   alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-
C_{10} cycloalkyl, R_2 is selected from the group consisting
of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_1-C_{10}
alkylamino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, thio, and C_1-
C_{10} alkylthio, and R_3 is selected from the group

15   consisting of R- and S- 1-phenylethyl, an unsubstituted
benzyl group, and a phenylethyl or benzyl group
substituted in one or more positions with a substituent
selected from the group consisting of C_1-C_{10} alkyl, amino,
halo, C_1-C_{10} haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10}

20   alkoxy, and sulfo.

34.   The method of claim 33, wherein R_1 is
$R^a R^b NC(=O)$, wherein R^a and R^b may be the same or different
and are selected from the group consisting of hydrogen,

25   C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl,
and C_3-C_{10} cycloalkyl, R_2 is selected from the group
consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino,

C_2-C_{10} alkenes, and C_2-C_{10} alkynes, and R_3 is selected from the group consisting of R- and S- 1-phenylethyl, an unsubstituted benzyl group, and a benzyl group substituted in one or more positions with a substituent
5   selected from the group consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl, nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo.

    35.   The method of claim 32, wherein the compound is
10   selected from the group consisting of N^6-benzyladenosine-5'-N-alkyluronamide-N^1-oxide and N^6-benzyladenosine-5'-N-dialkyluronamide-N^1-oxide.

    36.   The method of claim 32, wherein said compound
15   has the formula

wherein X is O or S, R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a and R^b may be the same or different and are selected from
20   the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and C_3-C_{10} cycloalkyl, or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10}
25   alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, R_2 and R_3 may be the same or different and are selected from the group consisting of C_1-C_{10} alkyl, R- and S- 1-phenylethyl, an unsubstituted benzyl group, and

160

a phenylether or benzyl group substituted in one or more
positions with a substituent selected from the group
consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo, and

5   R_4 is selected from the group consisting of halo, benzyl,
phenyl, C_3-C_{10} cycloalkyl, and C_1-C_{10} alkoxy.

       37.   The method of claim 36, wherein X is O, R_1 is
$R^aR^bNC(=O)$, wherein R^a and R^b may be the same or different

10   and are selected from the group consisting of hydrogen,
C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl,
and C_3-C_{10} cycloalkyl, R_2 and R_3 may be the same or
different and are selected from the group consisting of
C_1-C_{10} alkyl, R- and S- 1-phenylethyl, an unsubstituted

15   benzyl group, and a benzyl group substituted in one or
more positions with a substituent selected from the group
consisting of C_1-C_{10} alkyl, amino, halo, C_1-C_{10} haloalkyl,
nitro, hydroxy, acetamido, C_1-C_{10} alkoxy, and sulfo, and
R_4 is selected from the group consisting of halo, benzyl,

20   phenyl, and C_3-C_{10} cycloalkyl.

       38.   The method of claim 32, wherein said compound
has the formula

25

wherein R_1 is $R^aR^bNC(=O)$ or HOR^c, wherein R^a and R^b may be
the same or different and are selected from the group
consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10}

haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and C_3-C_{10} cycloalkyl, or are joined together to form a heterocyclic ring containing two to five carbon atoms, and R^c is selected from the group consisting of C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, and C_3-C_{10} cycloalkyl, and R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_1-C_{10} alkylamino, C_2-C_{10} alkenes, C_2-C_{10} alkynes, thio, and C_1-C_{10} alkylthio.

39.   The method of claim 38, wherein R_1 is $R^a R^b NC(=O)$, wherein R^a and R^b may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, C_1-C_{10} boc-aminoalkyl, and C_3-C_{10} cycloalkyl, and R_2 is selected from the group consisting of hydrogen, halo, C_1-C_{10} alkylethers, amino, C_2-C_{10} alkenes, and C_2-C_{10} alkynes.

40.   The method of claim 39, wherein R^a is hydrogen and R_2 is hydrogen.

41.   The method of claim 40, wherein R^b is a C_1-C_{10} alkyl.

42.   The method of claim 41, wherein R^b is methyl or ethyl.

43.   The method of claim 40, wherein R^b is a C_3-C_{10} cycloalkyl.

44.   The method of claim 43, wherein R^b is cyclopropyl.

45.   The method of claim 40, wherein R^b is a C_1-C_{10} aminoalkyl.

162

46.   The method of claim 40, wherein R^b is a C_1-C_{10} aminoethyl.

47.   The method of claim 40, wherein R^b is a C_1-C_{10} boc-aminoalkyl.

48.   The method of claim 47, wherein R^b is boc-aminoethyl.

49.   The method of claim 40, wherein the compound is selected from the group consisting of 5'-N-aminoethylamino-carboxamidoadenosine and 5'-N-boc-aminoethylamino-carboxamidoadenosine.

50.   The method of claim 40, wherein R^b is hydrogen.

51.   The method of claim 32, wherein said compound is a 1,3-R_1R_2-xanthine-7-riboside, wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl, and C_3-C_{10} cycloalkyl, or are joined together to form a heterocyclic ring containing two to five carbon atoms.

52.   The method of claim 51, wherein said compound is a 1,3-R_1R_2-xanthine-7-riboside, wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl, and C_3-C_{10} cycloalkyl.

53.   The method of claim 52, wherein said compound is a 1,3-dialkylxanthine-7-riboside.

54.   The method of claim 52, wherein said compound is 1,3-dibutylxanthine-7-riboside.

55. The method of claim 32, wherein said compound
is a $5'-R_3-1,3-R_1R_2$-xanthine-7-riboside, wherein R_1 and R_2
are selected from the group consisting of hydrogen, C_1-C_{10}
alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl,
and C_3-C_{10} cycloalkyl, or are joined together to form a
heterocyclic ring containing two to five carbon atoms,
and R_3 is selected from the group consisting of C_1-C_{10}
alkoxycarbonyl and aminocarbonyl, wherein the amino group
is unsubstituted or substituted at one or more positions
with a C_1-C_{10} alkyl.

56. The method of claim 55, wherein said compound
is a $5'-R_3-1,3-R_1R_2$-xanthine-7-riboside, wherein R_1 and R_2
are selected from the group consisting of hydrogen, C_1-C_{10}
alkyl, amino, C_1-C_{10} haloalkyl, C_1-C_{10} aminoalkyl, benzyl,
and C_3-C_{10} cycloalkyl, and R_3 is selected from the group
consisting of C_1-C_{10} alkoxycarbonyl and aminocarbonyl,
wherein the amino group is unsubstituted or substituted
at one or more positions with a C_1-C_{10} alkyl.

57. The method of claim 56, wherein said compound
is a $5'-R_3-1,3$-dibutylxanthine-7-riboside, wherein R_3 is
selected from the group consisting of C_1-C_{10}
alkoxycarbonyl and aminocarbonyl, wherein the amino group
is unsubstituted or substituted at one or more positions
with a C_1-C_{10} alkyl.

58. The method of claim 57, wherein said compound
is 5'-methylaminocarbonyl-1,3-dibutylxanthine-7-riboside.

59. The method of claim 51, wherein said compound
is chronically administered.

60. The method of claim 55, wherein said compound
is chronically administered.

164

61. The method of claim 58, wherein said compound is chronically administered.

62. The method of claim 32, wherein said mammal has or is at risk of having a condition, disorder, or disease state associated with the cellular release of inositol-1,4,5-triphosphate or diacylglycerol.

63. The method of claim 32, wherein said mammal has or is at risk for hyperactivity and said compound in binding to said A_3 adenosine receptors functions as a locomotor depressant.

64. The method of claim 32, wherein said mammal has or is at risk for hypertension and said compound in binding to said A_3 adenosine receptors functions as a hypotensive agent.

65. The method of claim 32, wherein said mammal has or is at risk for anxiety and said compound in binding to said A_3 adenosine receptors functions as an anxiolytic agent.

66. The method of claim 32, wherein said mammal has or is at risk for cerebral ischemia and said compound in binding to said A_3 adenosine receptors functions as a cerebroprotectant.

67. The method of claim 32, wherein said mammal has or is at risk for seizures and said compound in binding to said A_3 adenosine receptors functions as an antiseizure agent.

68. An assay which comprises providing a compound of claim 1 which has been labeled, contacting a sample with said labeled compound under conditions sufficient to

effect binding between said labeled compound and a component of said sample, and determining whether said binding occurred.

5   69.   An assay which comprises providing a compound of claim 24 which has been labeled, contacting a sample with said labeled compound under conditions sufficient to effect binding between said labeled compound and a component of said sample, and determining whether said
10   binding occurred.

      70.   An assay which comprises providing a compound of claim 25 which has been labeled, contacting a sample with said labeled compound under conditions sufficient to
15   effect binding between said labeled compound and a component of said sample, and determining whether said binding occurred.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

3/4
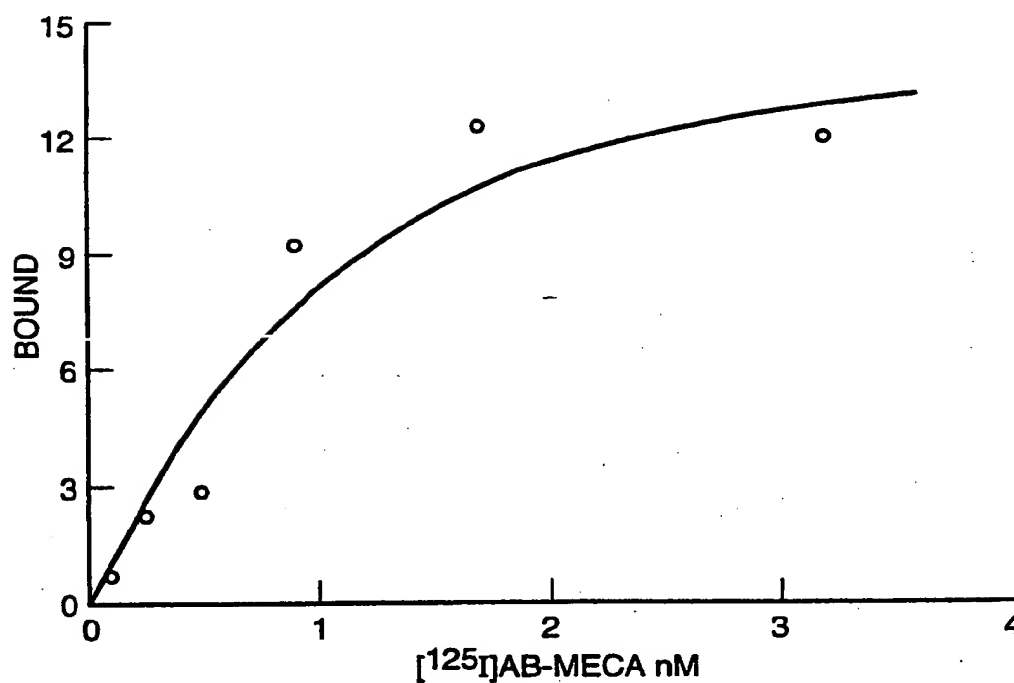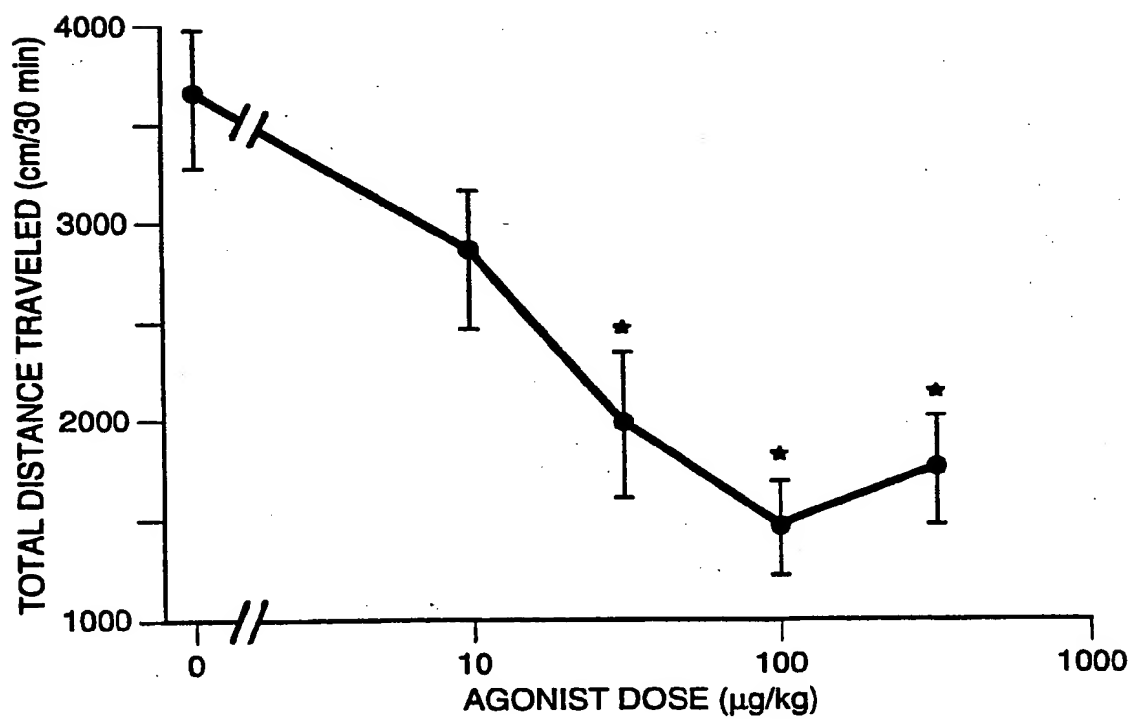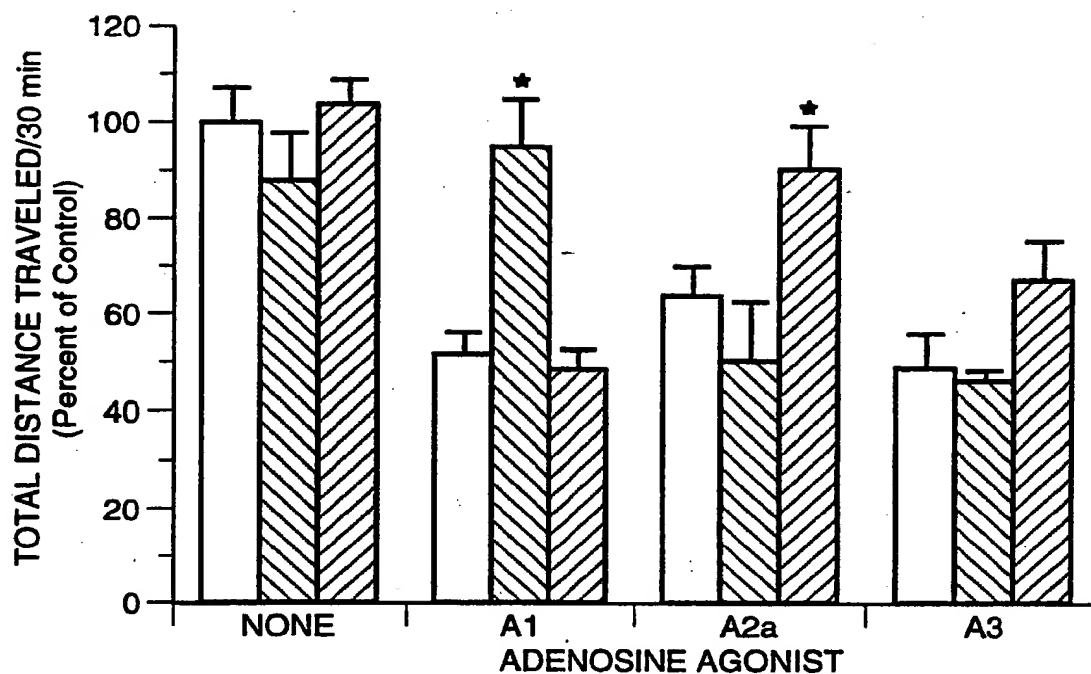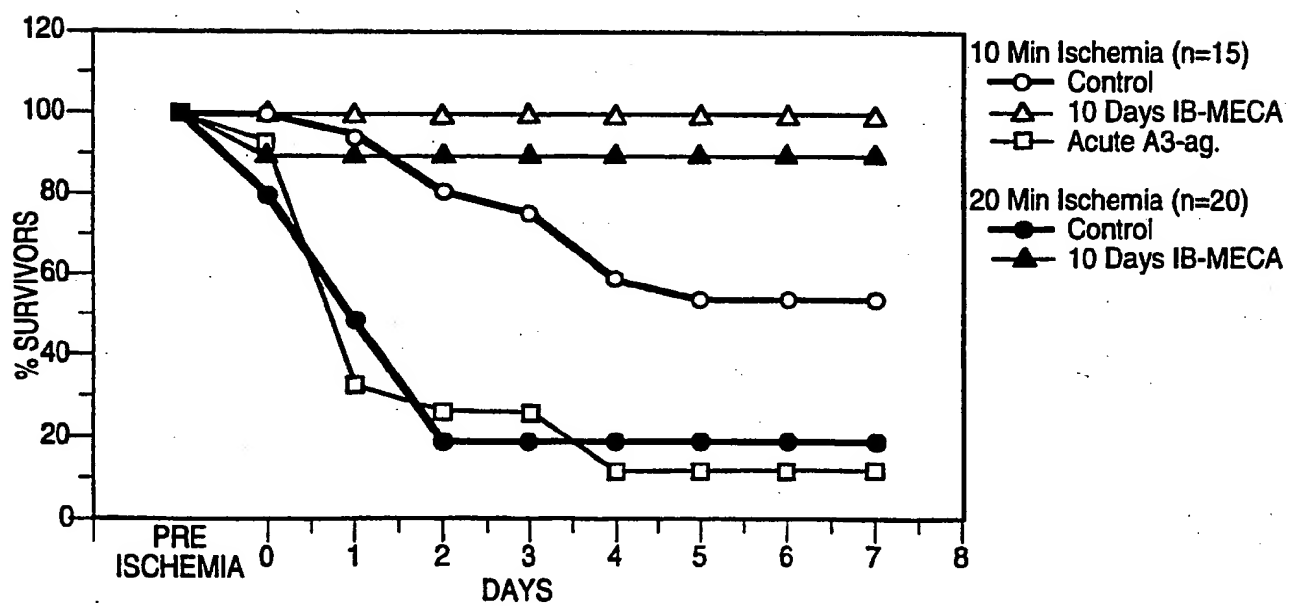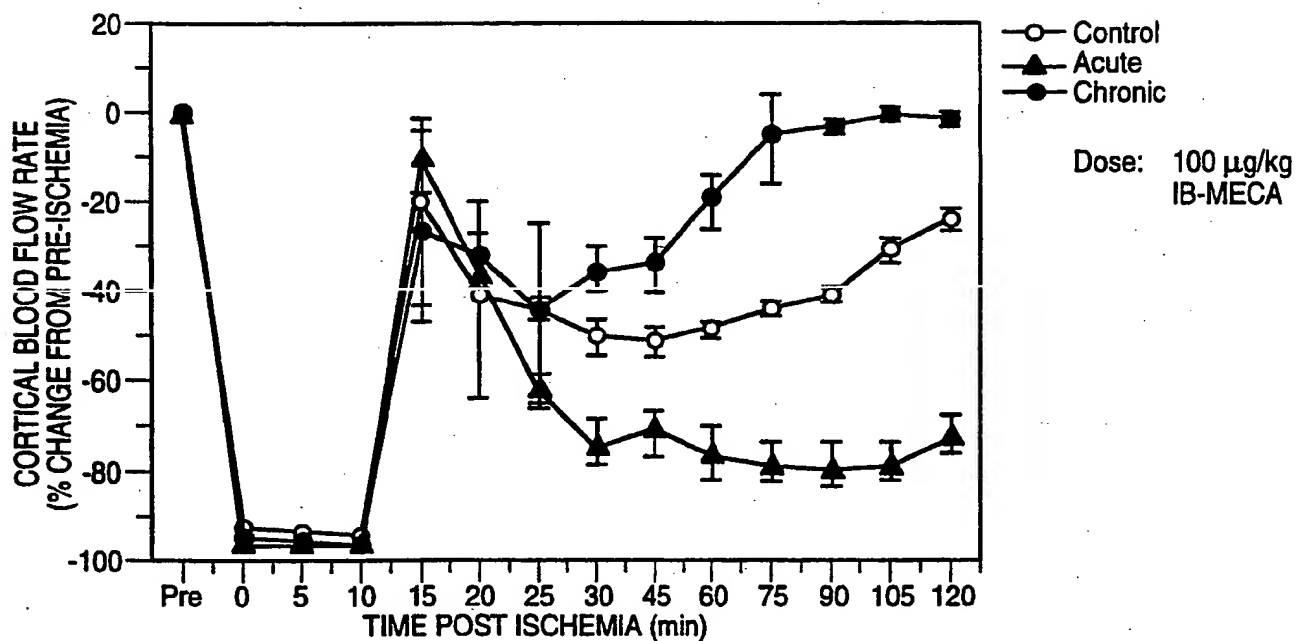
FIG. 5
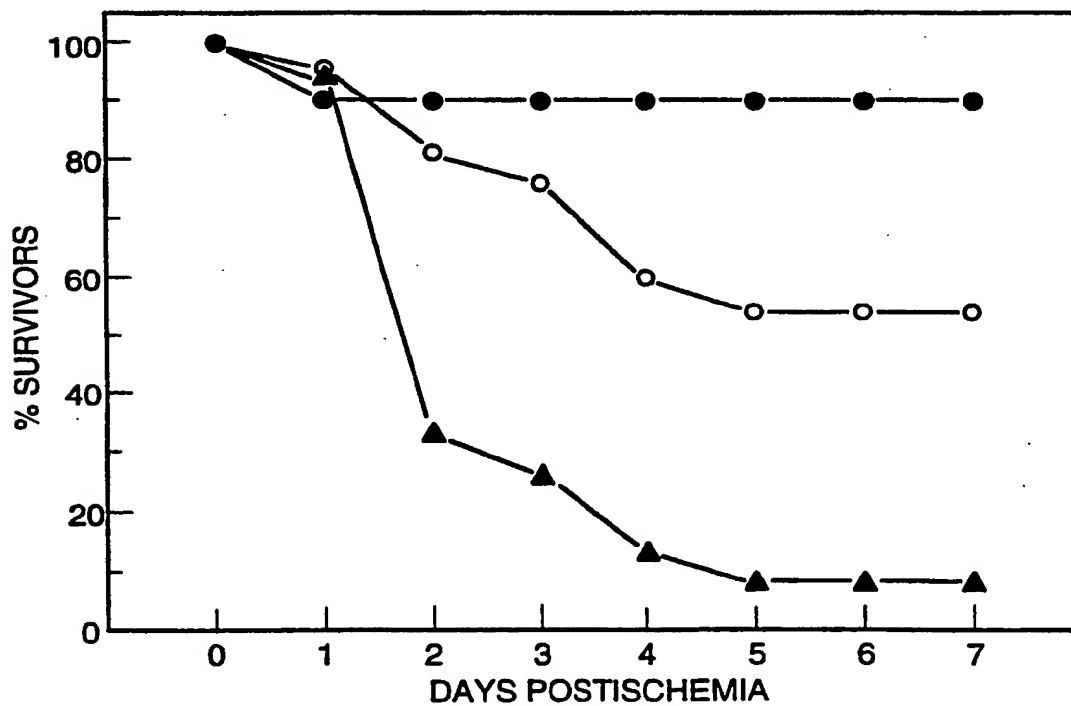
FIG. 6
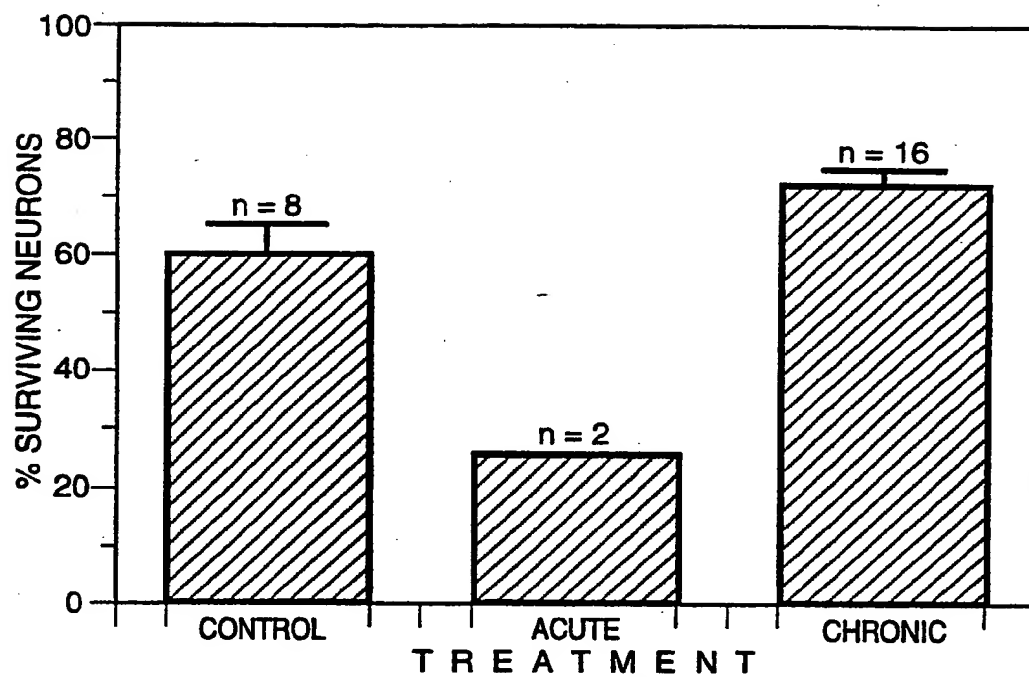
FIG. 7
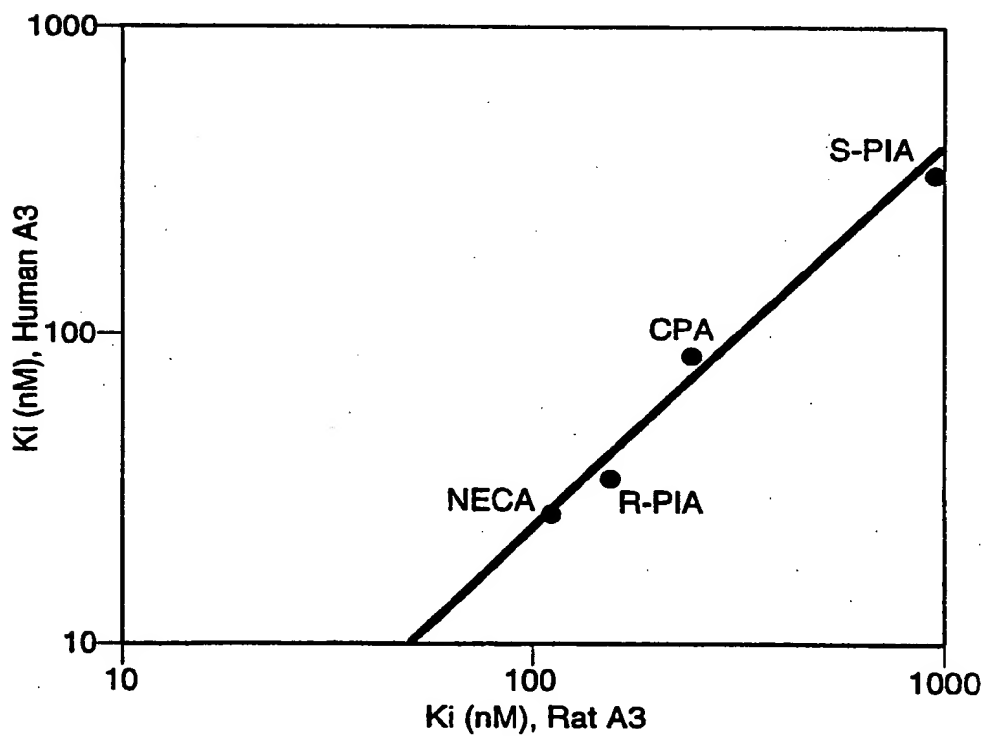
FIG. 8

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07H19/167   A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07H   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,86 00310 (NELSON RESEARCH AND DEVELOPMENT COMPANY) 16 January 1986 See the Whole Document, but especially, compounds 23 and 25 on page 11, compound 21 on page 12, compounds 35,36 and 38 on page 15 ---	1-4, 9-15,17
X	DE,A,25 24 284 (BOEHRINGER MANNHEIM GMBH) 28 October 1976 see page 4; examples 1,1a,and,1b --- -/--	1

| [X] Further documents are listed in the continuation of box C. | [X] Patent family members are listed in annex. |

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 1994	16. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Scott, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MEDICINAL CHEMISTRY, vol.35, no.3, 7 February 1992, WASHINGTON US pages 407 - 422 K.A.JACOBSON ET AL. 'Adenosine Receptors: Pharmacology, Structure-Activity Relationships, and Therapeutic Potential.' see examples 12-14	1
A	see the whole document	1-70
X	JOURNAL OF MEDICINAL CHEMISTRY, vol.29, no.6, June 1986, WASHINGTON US pages 989 - 996 S.KUSACHI ET AL. 'Dog Coronary Artery Adenosine Receptor: Structure of teh N6-Aryl Subregion.' See Table 1, compounds 14-31 and 47-63	1
X	CHEMICAL ABSTRACTS, vol. 72, no. 5, 2 February 1970, Columbus, Ohio, US; abstract no. 21921c, K.KOCH ET AL. '2-Aminoadenosine Derivatives with Cardiac Activity.' page 352 ;column 1 ; see abstract & SA,A,6 805 477 (BOEHRINGER, C.F., UND SOEHNE GMBH) 28 January 1969	1
X	CHEMICAL ABSTRACTS, vol. 70, no. 19, 12 May 1969, Columbus, Ohio, US; abstract no. 88212z, W.KAMPE ET AL. 'Adenosines' page 394 ;column 1 ; see abstract & SA,A,6 707 630 (BOEHRINGER C.F., UND SOEHNE GMBH) 21 December 1966	1
X	NUCLEOSIDES AND NUCLEOTIDES, vol.12, no.5, 1993 pages 431 - 439 MOSSHELI A.N. MOSSELHI 'Ribosylation of 8-Substituted Theophylline Derivatives.' See page 432, scheme 1, compound 7	25
P,X	JOURNAL OF MEDICINAL CHEMISTRY, vol.37, no.5, 4 March 1994, WASHINGTON US pages 636 - 646 C.GALLO-RODRIGUEZ ET AL. 'Structure-Activity Relationships of N-6-Benzyladenosine-5'-Uronamides as A3 Selective Adenosine Agonists.' see the whole document	1-17

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRITISH JOURNAL OF PHARMACOLOGY, vol.109, no.1, May 1993 pages 3 - 5 J.R.FOZARD ET AL. 'Adenosine A3 Receptors Mediate Hypotension in the Angiotensin II-supported Circulation of the Pithed Rat.' cited in the application see the whole document	1
A	NUCLEOSIDES AND NUCLEOTIDES, vol.10, no.5, 1991 pages 1191 - 1193 P.J.M.VAN GELEN ET AL. 'Xanthine-7-Ribosides as Adenosine Receptor Antagonists: Further Evidence For Adenosine's Anti Mode of Binding.' cited in the application see the whole document	25

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **[X]** Claims Nos.:   32-67
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 32-67 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. **[ ]** Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. **[ ]** Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **[ ]** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **[ ]** As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **[ ]** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. **[ ]** No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
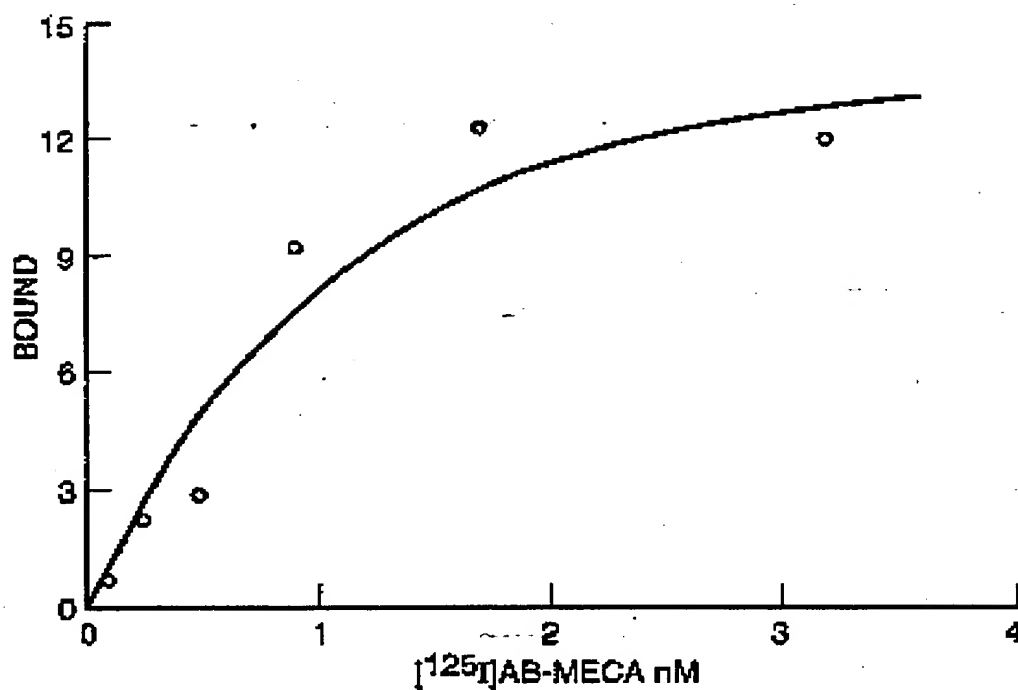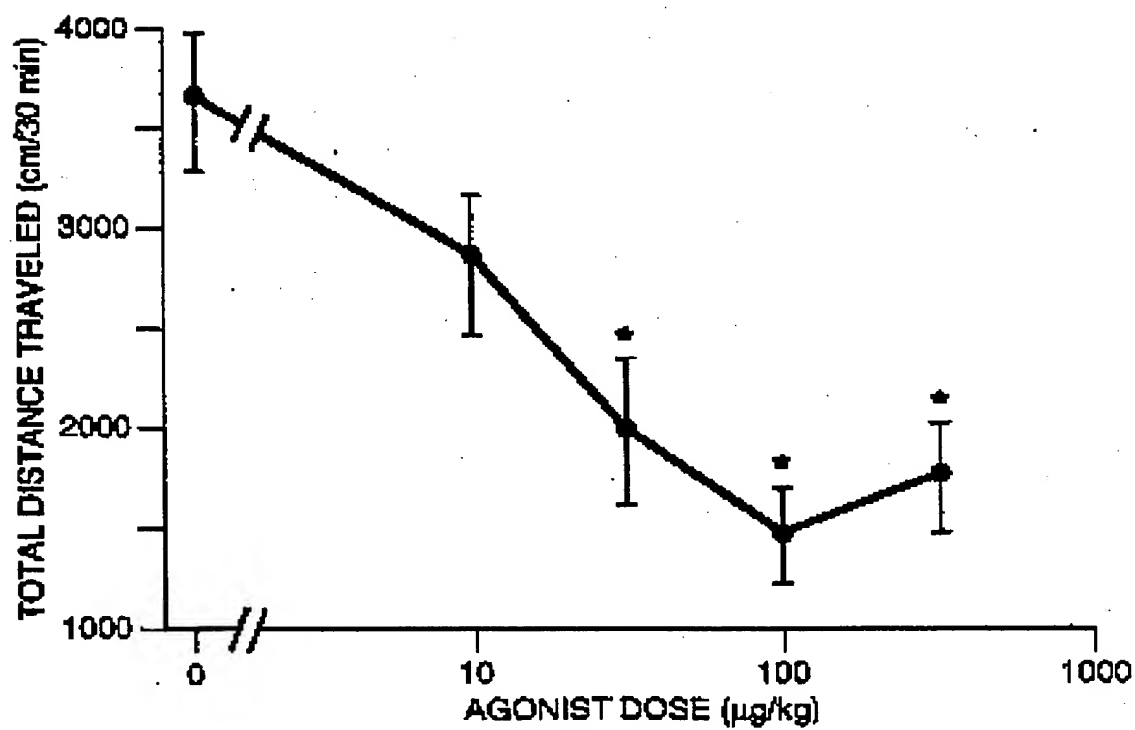
Remark on Protest   **[ ]** The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8600310	16-01-86	US-A-	5310731	10-05-94
		AU-B-	590724	16-11-89
		AU-A-	4497285	24-01-86
		EP-A,B	0191025	20-08-86
		JP-A-	61286398	16-12-86
DE-A-2524284	28-10-76	NONE		
SA-A-6805477		NONE		
SA-A-6707630		NONE		

1/4

FIG. 1

FIG. 2

2/4

FIG. 3

FIG. 4

FIG. 5

FIG. 6

4/4

FIG. 7

FIG. 8